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Extraction of the Hormone Necessary to Life from Adrenal Gland Substance.

By

Harold Tarver, B.Sc. (Alberta).

A thesis submitted from the Department of Biochemistry
in partial fulfilment of the requirements for
the degree of Master of Science at the
University of Alberta.

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1934
#25

The author is deeply indebted to Professor Hunter for his helpful criticism of this work during its progress.

The cooperation of Dr. M. M. Cantor who performed the adrenalectomies and treated the experimental animals is gratefully acknowledged. Protocols are taken from his data.

Introduction.

Anatomical and Embryological Considerations.

The adrenal glands, first described by Eustachius as early as 1563, consist in mammals of an outer cortical part surrounding an interior medulla.

The cortex which comprises the major portion of the gland can be further differentiated into three zones of cells called respectively the glomerular, fasciculated and reticulated zones, the last of which is in intimate association with the medulla. Frequently there are accessory cortical bodies located near the main body of tissue or in the genital region. This is particularly true in the case of the rabbit and rat.

Granules contained in the cortical cells are composed of fatty material. There is also a substance in these cells which is capable of reducing neutral silver nitrate in the absence of light.

In the cells of the medulla are granules of an entirely different nature, possessing the property of reducing salts of chromic acid, and of giving with ferric chloride a green colour. Such micro-chemical staining reactions are also possessed by cells associated with the sympathetic ganglia, in the carotid glands and in the abdominal chromaphil bodies.

The medulla and cortex of the adrenal gland are known to have different origins embryologically; the

cortex is developed from mesodermic tissue, whereas, the medulla is ectodermic in origin. It is also of interest to note that in cartilaginous fishes the two tissues exist separately. There are in these fishes a pair of suprarenal or chromaphil bodies quite separate from one or more interrenal bodies which correspond to the mammalian cortex. This suggests the possibility that there may be no direct physiological relationship between the two parts of the adrenal gland in mammals.

The Cortex as a Gland of Internal Secretion.

The first step towards the experimental elucidation of the function of the adrenal glands was made by Brown-Sequard (1856) who completely adrenalectomized many animals including rabbits, guinea pigs, cats and dogs. He observed that these animals died within a few hours and concluded that the glands were necessary to life. That the result of complete adrenalectomy is fatal has been amply verified on different species of vertebrates but many of the earlier experimental animals undoubtedly died from the results of the operative procedures employed rather than from adrenal insufficiency. Biedl (1913) was among the first to reduce the severity of the operation and to obtain a correspondingly lengthened post-operative survival. Unfortunately many recent workers have not given sufficient consideration to errors arising from

the severity of the post-operative symptoms after complete adrenal removal.

After bilateral adrenalectomy the major portion of the animal's cortical and medullary substance has been removed. It still remains uncertain whether the cortex, the medulla or both are essential to life. The careful experiments of Biedl on Elasmobranch fishes were instrumental in settling this particular point. This investigator removed the interrenal body in these fishes whereupon the animals died within three weeks showing the symptoms of typical adrenal insufficiency. Thus Biedl claimed to have shown the necessity of the cortex to life. Wheeler & Vincent (1) destroyed the medulla in a series of dogs and cats. In no case did any of these animals die except when a large proportion of the cortex was destroyed along with the medulla. Further experimental evidence pointing to the necessity of the cortex to life has been provided by the work of Houssay & Lewis (2), Wislocki & Crowe (3), Zwemer (4); and Rogoff (5) from studies in medullary function has also arrived at the conclusion that the cortex is the more vital part of the gland.

It may, therefore, be assumed that the cortex is necessary to life. We are led to believe it exercises this function by producing an internal secretion because

Jaffe (6) has transplanted adrenal cortical tissue homoplastically. In animals (guinea pigs) in which successful transplants were made cortical tissue maintained life indefinitely. Its removal resulted in death. Animals with functioning cortical transplants were normal in all respects.

Cortical Suppression.

Following adrenalectomy the symptoms of adrenal insufficiency appear after a variable number of days of apparently healthy life depending on the experimental conditions and the animal employed. These symptoms which are characteristic have been fully described by many authors. They are, in brief, first a loss of appetite, apathy, then muscular weakness in the hindquarters which rapidly spreads to all parts until the animal is totally prostrate. It is noteworthy that the blood pressure and body temperature both decrease as the severity of the symptoms becomes greater. Death follows inevitably but is sometimes preceded by convulsions similar to those resulting from hypoglycaemia. Because these animals refuse food they gradually lose weight until death supervenes.

It has been shown that extracts can be prepared from adrenal glands which will on daily injection maintain

such adrenalectomized animals in good condition indefintely.

Addison's Disease.

The condition of Addison's disease in man resembles in its main points the condition brought about by adrenalectomy in animals, if cognizance is taken of the fact that the operated animals are suffering from acute suppression of adrenal function, whereas, in man a chronic condition gradually develops. The clinical symptoms of this disease were first comprehensively described by Thomas Addison in 1855, who, at that time, associated the condition with lesions in the adrenal glands. The condition he described is characterized by muscular weakness, anorexia, low blood pressure, anaemia, gastro-intestinal symptoms, loss of weight and pigmentation of the skin, mucous membranes and conjunctiva. Generally the disease is associated with tuberculosis or atrophy of the adrenal glands. Addison's disease, however, has been observed in patients who proved at autopsy to have normal adrenal medullas and atrophy of the cortex alone. Thus it would appear that the disease results from hypofunction of the adrenal cortex.

We have, then, good evidence (a) that the adrenal gland produces an internal secretion besides adrenalin. (b) that by giving a substitutive treatment to adrenal-

ectomized animals, potency of extracts can be demonstrated. (c) The extracts may be useful in the treatment of Addison's disease.

Preparation of Extracts of the Gland.

Medulla.

In the year 1894, Oliver & Schäfer first observed that extracts of the adrenal medulla when injected intravenously caused a rapid rise in blood pressure. The action of these extracts was shown to be quite independent of the central nervous system.

Many experimenters, thereupon, started work on the medulla of the gland with the object of isolating the strongly reducing active principle in pure form. These researches were so successful that in 1901, Takamine and Aldrich, working independently, isolated a pure substance now known as adrenalin, which possessed the typical (physiological) properties of the tissue from which it was extracted. Within three years the structure of this compound was determined and its synthesis effected.

The properties of this substance isolated from the adrenal medulla were of such a striking nature that the attention of physiologists and chemists was directed towards the elucidation of its properties and functional significance. That injected adrenalin will not maintain

adrenalectomized animals has been amply demonstrated. Moreover, in the intact animal adrenalin has been shown to be confined to the medulla of the gland.

Since in patients with Addison's disease one of the symptoms is low blood pressure it might be supposed that this results from an insufficient supply of adrenalin passing into the blood stream. However, treatment of these patients with adrenalin is without permanent benefit.

Cortex.

Many early experimenters attempted to keep adrenalectomized animals alive by employing aqueous or saline extracts of the whole gland or cortex but it was not until lipoid solvents were adopted that any certain degree of success was attained. Potent extracts were prepared by Swingle & Pfiffner (7,8,9) and Hartman et al (10) employing such solvents for the preliminary extraction, and all potent extracts have been prepared using such extracting agents. For convenience,.the various methods of extraction which have been developed will be dealt with according to the nature of the first extractive employed.

1. Alcohol.
2. Ether.
3. (a) Acetone,
   (b) Acidified acetone.

(1) Swingle & Pfiffner (8,9,19) prepare extracts as

follows.

The hormone is extracted from the minced whole glands or cortex with a large volume of 95% alcohol. The gland substance is removed and the alcohol completely distilled off under reduced pressure. The remaining aqueous phase is then extracted with benzene, the benzene removed by distillation at low temperature and the sticky residue exhausted with cold acetone. After removal of this acetone the residue is distributed between low boiling petrol ether and 70% ethyl alcohol to remove sterols. The substance is removed from the 70% alcohol and introduced into 95% alcohol which is then percolated through a column of Permutit to remove adrenalin. The residue in the 95% alcohol is introduced into aqueous solution, and clarified using a Seitz filter.

A second extract containing an additional portion of the hormone is made by extracting the gland residue with 80% alcohol and proceeding as with the first extract.

(2) A second method of preparing extracts is that of Hartman. This method has been recently described in detail (11).

The minced cortex is extracted by shaking in the absence of light, with ether, the process being repeated three times in all. The ether extracts are concentrated to a fatty residue, which is thoroughly extracted with

warm 95% alcohol. The alcoholic extracts are reduced to 80% by the addition of water, cooled and the precipitate removed in the cold. The inactive precipitate is reextracted following which the substance is introduced into isotonic saline. The method has been reported on unfavourably by Britton, and others, yet one cannot doubt Hartman's extracts do contain demonstrable quantities of the hormone.

(3) Kutz (12) has prepared adrenal extracts by employing acetone as the first solvent.

Whole glands are extracted with absolute acetone, the acetone is removed from the extract under reduced pressure and the aqueous solution is treated to obtain a benzene extract as in the Swingle & Pfiffner method. The combined benzene extracts are concentrated and the concentrate washed free from adrenalin with 4% sodium bicarbonate. The benzene extract is then introduced into aqueous solution from which the lipoid is removed by adding excess of acetic acid. The filtrate from this solution is employed after partial neutralization.

Grollman and Firor (13) have developed the above method so that they use a larger volume of acetone per unit of gland substance together with a freezing thawing procedure during an extraction made with or without refluxing. They do not remove this acetone completely

before extracting the residue with benzene. Seven extractions with benzene in place of the four or five used by other workers are employed. Without concentration adrenalin is removed from the extract by washing once or twice with cold saturated sodium bicarbonate, following which the benzene solution is treated with a small volume of normal hydrochloric acid to neutralize the alkali. This extract after removal of the benzene and acetone is introduced into saline and Seitz filtered for use.

(4) Kendall & MacKenzie (14) have employed acidified acetone as the initial extracting agent.

These workers concluded that only a small fraction of the hormone is removed from the glands employing neutral reagents and that addition of sulphuric acid to the extracting acetone to pH3 allows of a more complete removal of the hormone. After a comparatively long extraction period the acetone sulphuric acid is filtered off, the acetone removed under reduced pressure and the watery extract precipitated with lead acetate at pH5.5. The filtrate freed from lead is further purified by extracting a dry residue with acetone and methyl alcohol. Adrenalin is removed by treating a methyl alcohol solution with lead nitrate and sodium bicarbonate. On introduction into water this extract is ready for use.

It is of interest to note at this point that the

hormone or a substance resembling the hormone in its physiological effect on adrenalectomized animals is extractable from urine by employing comparable processes (15, 16).

These various methods of extraction are in principle essentially the same (with the exception of Kendall's method). They involve the following assumptions being made as to the properties of the hormone.

The substance in situ is soluble in ethyl or methyl alcohol, or acetone either 100% or more dilute, soluble in water or saline after the removal of fats and other substances, soluble in benzene and ether at least in the presence of fat, relatively insoluble in low boiling petrol ether (hexane), stable to treatment with sodium bicarbonate solution and mineral acid, does not displace the sodium in Permutit nor is it adsorbed on this reagent to any considerable extent.

All the described methods of extraction lead to products which will maintain or at least extend the life-spans of adrenalectomized animals. Other criteria of potency have been employed by certain workers whose results are difficult to evaluate. Schmitz & Kuhnau (17), and Loewe et al (18) may be mentioned.

Investigation of the Extraction and Extracts.

Pfiffner and coworkers, and Harrop have shown that

much activity is extractable from medullary as well as from cortical substance. Pfiffner (19) gives the following figures,

	Whole Gland.	Dissected Cortex.
Weight of material	1000	720
Dog units/cc.	40-40	4-10
Total yield of hormone	1000-2000	96-240,

whereas Harrop finds as a result of extracting equal weights of different tissues dissected out and immediately placed in 95% alcohol,

	Dog Units/cc.
Cortical tissue from periphery without any medullary tissue.	less than 3
Ordinarily prepared cortical tissue.	8
Medullary tissue.	8-10
Whole gland tissue.	15-20.

Swingle & Pfiffner (21) further report that freezing the gland substance for five months prior to extraction does not decrease perceptibly the yield of hormone obtainable from them. Autolysis at room temperature for a period of two days also does not affect the yield of hormone.

Swingle & Pfiffner's work indicated that extracts are most stable in benzene and less stable in alcohol-water mixtures. Stability in aqueous solution may be increased by the addition of benzoic acid.

Pfiffner, Vars, Bott & Swingle (22) further purify hormone preparations by washing etherial solutions of the

hormone with 0.5 N. hydrochloric acid. The hormone is extracted quantitatively by the acid employed. Treatment of the hormone solution with 0.05 N. aqueous alkali results in complete destruction of the activity formerly present.

These workers have employed another method in fractionation. They are able to precipitate inactive material from absolute alcoholic solutions of the hormone by the addition of a large volume of hexane.

As to the chemical properties of their extract Swingle & Pfiffner (23) have reported the following tests as negative, biuret, ninhydrin, Hopkins-Cole, Molisch, Pauly, Knoop (Hunter's modification), Liebermann-Burchard; and positive reactions with the following, Millons, xanthoproteic, alkaline copper, alkaline phosphotungstate.

The Assay of Adrenal Gland Extracts.

The original investigators, Swingle & Pfiffner and Hartman and his associates, assayed their various extract preparations by employing adrenalectomized cats. Both these workers were able to show that cats without demonstrable accessory cortical tissue, could be kept alive by daily subcutaneous injections of active preparations in sufficient amount. Such treated cats were indistinguishable from intact animals in their behaviour. On

the cessation of extract administration the cats survived only a limited number of days.

More recently, the majority of researchers have employed other animals such as dogs, rats, and rabbits preferring them to cats for experimental purposes.

For many years it has been generally believed that the rats survive complete adrenal removal without more than transitory symptoms of insufficiency. Nevertheless, considerable evidence has recently been presented which shows that such is not the case (12,24,25). Adrenalectomy is just as fatal in the young rat as in any other species provided the operative removal is performed with thoroughness. It is of advantage to use rats for purposes of assay rather than dogs if this is possible. The rat is a very hardy animal and is much more resistant to operative shock and to any intercurrent infection that is liable to kill other experimental animals.

Kutz (12) has, therefore, developed a method of testing the potency of adrenal extracts using adrenalectomized one month old rats. The extract is given to the rats subcutaneously for a period of at least twenty days following operation. Rats treated with potent extracts grow normally during treatment.

Grollman & Firor (13) use a very similar method to assay their preparation but continue extract treatment

for a period of seven days following the adrenalectomy. Cessation of treatment results in the animals dying within a few days.

Torino & Lewis (26) have shown that adrenalectomized rats are killed by doses of morphine non-fatal to normal animals. Injection of potent extracts of the adrenal cortex for three days before giving the morphine to such animals protects them against its abnormally toxic effect. This observation has been developed by these workers into a method of assay. The method, nevertheless, does not appear to possess any distinct advantage over that of Grollman & Firor for it likewise necessitates the use of adrenalectomized animals.

The following tables gives the post-operative survivals of the two species with which we are concerned.

Table 1.

Post Operative Survival of Adrenalectomized Cats in Days.

Worker	Number of Animals	Average Survival	Range of Survival	Operation
Swingle & Pfiffner(7)	38	7.7	4.15	two stage
Swingle(27)	100	8.6		"
Rogoff & Stewart(28)	26	11	5-20	two stage
	20	10.7	6-30	"
Marine & Baumann(29)	18	5.2	2-12	one stage
Britton(31)	20	7.5	3-11	one stage
Cantor*	10	6.3	1.5-11	one or two stage

*Several of the cats included were treated with impotent extracts.

Table 11.

Post Operative Survival of Adrenalectomized Rats in Days.

Worker	Number of Animals	Age or Weight	Average Survival	Range of Survival	Per cent Mortality
Kutz(12)	57	28 days			98
Firor & Grollman (25)	6	50 gm.	1		100
	14	50-75gm.	2	1-5	100
	38	76-100gm.	4.3	1-9	100
	100	over 100gm.	7.9	1-21	82
Pencharz, et al (24)	62	70-358 gm.	8.3	2-18	100
Gaunt(30)	130	35-280 days	7	1-20.5	95
Cantor**	28	40-95 gm.	4.5	1.5-8	89

**Taken from the Control Rats included in the protocols.

Experimental Part.

Extracts have been prepared according to the Swingle & Pfiffner, Kutz, Kendall & MacKenzie, and Grollman & Firor methods, from whole adrenal glands obtained locally. The glands are frozen soon after excision and stored until required for use. Usually they are delivered frozen and are treated immediately. Various modifications of the four methods have been tried.

Originally the extract preparations were tested on adrenalectomized cats, but more recently adrenalectomized rats have been used exclusively. Dr. M. M. Cantor is responsible for the operations and treatment of the animals. As opportunity has presented itself extracts have also been provided for the treatment of patients with Addison's disease. The experimental work is accordingly reported in three parts according to the disposition of the individual extracts.

A. Extracts tested on cats.
B. Extracts tested on rats.
C. Extracts provided for clinical use.

Where possible, correlations between, and ambiguities in results will be pointed out.

A. Extracts Tested on Cats.

All the extracts tested on these animals were prepared according to a slightly modified Swingle & Pfiffner

technique. However, in some cases, instead of employing alcohol for the preliminary extraction, acetone was used. To illustrate the process the preparation of a typical lot (No 62) is described. The description is based on 1 kgm. gland substance.

After partial thawing out, the gland substance was minced twice, the second time into 2.5 L. of 95% ethyl alcohol contained in an enamel tub. The mixture was well stirred then, and occasionally in the subsequent 24 hour extracting period.

The alcoholic extract was removed from the gland residue by a process of expressing through cheese cloth in a hand press and filtration through soft filter paper. Thus a 'dry' residue was obtained which, after being reminced, was extracted with 1.0 L. of 82% alcohol for a period of 30 hours. After this time the second alcoholic extract was removed from the gland residue as before.

The alcohol was distilled from both extracts under reduced pressure at a temperature which at no time exceeded 50°C. The first alcohol was concentrated to one fourteenth of its original volume and the second to a thirtieth. In the combined aqueous solutions there was considerable suspended solid material, most of which was derived from the second alcoholic extract, but both liquid and solid were extracted with benzene four times.

The fourth benzene wash was almost colourless. For each extraction 80 cc. of benzene were employed. At this point the aqueous solution was discarded.

Next day the combined benzene extracts were concentrated to a small volume under reduced pressure so that the distillation temperature never exceeded 45°C. The viscous concentrate obtained was poured slowly into 220 cc. of cooled and agitated acetone. After standing several hours the precipitated phospholipin was filtered off.

The acetone was removed with the precautions previously observed and the resultant residue was dissolved and distributed between petrol ether and 70% alcohol in a separatory funnel. Ten cubic centimeters of the petrol ether and 22 cc. of alcohol were employed. The petrol ether was removed and the alcoholic solution washed three more times with 10 cc. portions of the ether. The four petrol ether solutions were then extracted with a 5 cc. portion of 70% alcohol starting with the fourth and treating the extracts in reverse order with the one portion of alcohol. The two 70% alcoholic extracts were combined and the four petrol ether solutions discarded.

The 70% alcoholwas removed at a low distillation temperature and the almost dry residue introduced into 95% alcohol, 15 cc. After standing for a period of four

days the extract was filtered from a dark brown precipitate which had appeared in the interim. The alcoholic solution was then shaken for one hour with 2.2 gm. Permutit, (a synthetic zeolite). The Permutit was filtered off and washed with a small volume of alcohol (95%). Then, because, the extract still contained an amount of adrenalin greater than permissible as shown by a test with the uric acid reagent (Folin-Denis) the alcohol was treated similarly with 1.5 gm. Permutit. Following this the Permutit was removed and washed with alcohol.

The alcohol was then distilled off under reduced pressure in the usual manner, water being added towards the end of the distillation. Thus the material was gradually introduced, insofar as possible, into aqueous solution. After standing several hours in the refrigerator the solution was Seitz filtered to clarify and sterilize it. The aqueous solution was made up to a final volume of 10 cc., that is, the equivalent of 100 gm. of whole gland substance per cc. of extract.

When not in process of manipulation extracts, except for the first alcoholic solutions, have always been kept in the refrigerator maintained at 0°-4°C.

All solvents have been distilled from the extracts at low temperature and in no case has an extract been heated above 55°C. Although the extracts sometimes foam

quite badly the distillation temperature has usually been maintained between 30° and 40°C. throughout the process.

To facilitate distillation, particularly of solutions containing a large percentage of water, or benzene only, natural gas washed by bubbling through concentrated sulphuric acid and alkaline pyrogallate, or carbon dioxide, or air was led through the solution. There appears to be no advantage in using gases other than air.

A number of extracts were made according to the method of lot 62. For the initial extraction either alcohol or acetone was used.

The preparations made using acetone all involve extraction of the minced glands overnight, followed by washing the gland residue with a second portion of 80% acetone. Instead of preparing the acetone extract from benzene solution as done in the case of lot 62, by precipitation, a dry benzene residue was extracted with acetone several times (wash method).

These preparations are summarized in Table 111.

Table 111.

Extracts Prepared Using Acetone. Basis 1 kgm. glands.

Lot Number	50	51	53	63A
1. Litres 1st acetone	1.9	1.9	2.0	2.5 at pH3
2. Litres 2nd acetone	0.63	0.42	0.55	0.75
3. Litres aqueous concentrate	0.21	0.18	0.23	0.22
4. Times benz. sol. washed with 4% Na_2CO_3	7	5	5	
5. Times benz. sol. washed with H_2O	3	3	2	
6. Method of obt. acetone extract	wash	wash	wash	pptn.
7. Petrol ether washes	2	3	4	4*
8. Times shaken with Permutit	1	1	1	4
9. Gm. Permutit used	3.2	2.1		11.9
10. Gm. total residue	0.018	0.020		
11. Cats used by number	23,28 29,30	28,29, 30	38	52

*Petrol ethers washed back with 70% alcohol once.

In these acetone extracts no appreciable potency was demonstrable. The lack of success may be attributed to a combination of four factors, namely, low potency in the extracts, inadequate dosage, tardiness in extending therapeutic measures after complete adrenalectomy, and the presence of infections in some of the treated animals. The only cat (38) in which the treatment appeared to be at all effective was maintained on massive doses of extract, namely the equivalent of 1,000 gm. whole gland substance per day, and treatment was begun immediately following the operation. Notwithstanding this dosage, the cat began to show signs of failure on the eleventh day after operation, dying on the seventeenth day.

These extracts were washed with 4% sodium carbonate solution. Significant amounts of activity may have been destroyed by this procedure.

There likewise, appears to be little potency in the extract prepared using acidified acetone as the extracting medium (lot 63A, cat 52), although the experimental evidence for this conclusion is hardly adequate.

Table lV. gives the details of a group of extracts prepared using alcohol as the initial extracting agent. The minced glands were extracted with the indicated volumes of alcohol overnight and then with second portions of alcohol (80-90%) for the same length of time.

Table lV.

Extracts Prepared Using Alcohol. Basis 1 kgm. glands.

					60		
Lot Number	56	57	58	59	60A	60B	62
Litres 1st alcohol	1.7	2.4	2.4	2.4	2.4	2.4	2.4
Litres 2nd alcohol		1.5	1.1	1.2	1.0	1.0	1.0
Litres aqueous concentrate	0.20	0.41	0.96	0.49	0.43	0.43	0.21
Petrol ether washes	5	5	4	4	4	6	4
Times shaken with Permutit	2	2	1	2	3	1	2
Gm. Permutit used	4.9	3.0	2.9	5.0	2.8	3.3	3.7
Mgm. total residue		45	47	72			62
Cats used by number	23, 38, 39	38, 40, 44	40, 44, 46	40, 44, 46	40, 46	40, 46	52, 54, 55

Extracts prepared using alcohol as the first solvent were more successfully employed to prolong the lives of cats 40, 52, and 54. An anomalous result was obtained with cat 55 which died in spite of a similar type of extract treatment. Treatment of the cats maintained on these extracts was commenced either immediately after bilateral adrenalectomy or two days before the removal of the second gland. The results obtained are therefore not strictly comparable with the results obtained with the previous group of cats in which therapeutic measures were initiated later.(cat 38 excepted).

The potency of lots 56-59 appears to be quite small and only slightly better than that found in the acetone extracts, that of lots 60 and 62 is greater. It is possible to correlate the potency of these extracts with the nature of the petrol ether-70% alcohol distribution stage. When comparatively large volumes of petrol ether were used and the petrol ether extracts were not washed back with 70% alcohol, loss of potency evidently occurred. This point is illustrated by the following table which provides the details of the distribution stages and an arbitary estimate of potency.

Table V.

Details of the 70% Alcohol-Petrol Ether Distribution Stages in Lots 57-62.

Lot Number		57	58	59	60A	60B	62
Cc. 70% alcohol	1.	22	29	29	20	20	22
Cc. Petrol ether	1.	14	14	20	10	10	10
	2.	9	11	14	10	10	10
	3.	7	9	14	9	9	10
	4.	7	6		9	9	10
	5.	7				4	
	6.					3	
Cc. 70% alcohol	2.						5
Potency		poor	better than 57 or 59	poor	fair	poor	quite good

From these results there is an indication that the hormone is appreciably soluble in petrol ether under the conditions obtaining in the solution.

It is notable also (cf. Tables 111 & 1V) that the residues from the alcoholic are greater than those from the acetone extracts.

B. Extracts Tested on Rats.

(1) Swingle & Pfiffner method using alcohol or acetone.

Some of the extracts (56,62) which were tested on the cat series were also tried on rats and other extracts, prepared following similar methods, have been used for the same purpose. These preparations are summarized in the following table.

Table Vl.

Extracts Prepared Using Alcohol (54) or Acetone (55-70B).
(Basis 1 kgm. glands).

Lot Number	54	55	60Bb	63B	64	678 C	 D	70B
1. Litres 1st agent	2.0	2.0	2.5	2.4	2.2	2.4		2.7
2. Days extracted	1	2	1	1	1	3		3
3. Litres 2nd agent	0.60	1.0	1.0	0.75		1.9		1.5
4. Days extracted	5hrs.	1hr.	1	4hrs.		3		3
5. Litres aqueous concentrate	0.37	0.63	1.00	0.22	0.28	0.27		0.40
6. Method of obt. acetone ext.	Wash	Wash	Two Pptns	Pptn.	Pptn	Two Pptns.		Pptn & Wash
7. No. distbns. (70% alch.)	1	1	1	1*	1*	5	5	5
8. Petrol ether washes	4	4	8	4	4	25	25	
9. Permutit method		S.	S.	S.	S.	P.	P.	P.
10. Times treated with Permutit		2	6	2	2	2	1	1
11. Gm. Permutit used		5.0	5.0	5.5	4.7	15	15	12.5
12. Mgm. total residue	3.3	28	13	43	18	54	54	62
13. Rats used by groups number	5,6	8,9 10,11	14, 15A	15B, 16B	16A	19B	19C	21

*Petrol ethers washed back once with 70% alcohol as lot 62.
S., shaking; P., percolation.

Lot 678 is made up of extracts 66, 67, and 68. These lots were combined after getting rid of the phospholipin and stored in benzene. Out of the combined lots aliquots were taken to prepare extracts 678C and 678D.

The three lots of gland substance used were extracted for a longer period of time than usual (3 days). This resulted in the production of very dark coloured initial alcoholic extracts which contained considerable toxic material. To remove this material it is necessary to employ larger amounts of Permutit thanwere used previously.

Similarly when benzene extracts of gland substance after removal of phospholipin were allowed to stand two months in the refrigerator the adrenalin content gradually became less but toxic material appeared in its place.

Starting with lot 64 the method of extract preparation was changed in the petrol ether and Permutit stages.

The distribution stage between 70% alcohol and petrol ether was elaborated in order to minimize the loss of activity in the discarded petrol ethers. After each distribution the five petrol ethers were taken down nearly to dryness and the residue redistributed between a fresh lot of 70% alcohol and petrol ether. This distribution procedure was repeated five times in all to obtain five 70% alcohol solutions instead of the previous one or two. The residue determinations show that even the last of these 70% alcoholic solutions contains a considerable proportion of the total solid. Very stable emulsions

were often produced if the two phases were shaken too violently together. It was found inadvisable to dissolve the residue in petrol ether and 95% alcohol and then add water to the solution to give 70% alcohol. When this was done no separation into two phases took place.

The Permutit stage was changed so that instead of using a shaking procedure percolation was employed.

The extracts (678 & 70B) were percolated through a column of Permutit contained in one or more glass tubes of 1.7 cm. internal diameter, with 10 gm. of the reagent in a length of 9 cm. With the aid of suction the speed of percolation was adjusted so that one or two drops of extract went through the column each second. Before and after percolation the column was washed out with alcohol and for each extract the glass tubes were refilled with fresh Permutit.

The first extract like those prepared for cats, was made using acetone as the initial solvent. It was not adequately tested since the treatment of the experimental animals was only commenced after the appearance of insufficiency symptoms. The symptoms were not alleviated so the animals all died without their life spans being extended beyond that of the control animals, e.g. group 6.

Extracts prepared using alcohol as the initial solvent were employed with more success. It is doubtful

whether this was due to any great increase in potency of the extracts for the results may be, alternatively, attributed to the improvement in the treatment of the experimental animals. Extract treatment was found to be most successful if commenced within one or two days after bilateral adrenalectomy.

Many of the rats adrenalectomized at this date were of a comparatively large size. Since such animals survive longer than small animals and more of them continue to live indefinitely after operation, no safe deductions can be drawn from these early studies. However, during this time a better technique was gradually developed.

The rats on extract 55 were the first to show any certain response. Rats from subsequent groups responded to greater or less degree to lots 60Bb, 62, 63B, 64(?), 678C, 678D, 70B.

The response to extract 63B, is rather surprising because 63A, was apparently inactive (cat 52). These extracts represent two parts of the one lot, one of which was treated with large amounts of Permutit (A) to remove toxic material, whereas the other was treated with Lloyds reagent (B) in alcohol for the same purpose.

Two dosage levels were tried in the case of lot 678C. At a dosage of 34 gm. of whole gland substance per day the rats showed a slight response but when this

dosage was doubled three out of four rats remained alive indefinitely after discontinuing treatment. Very similar results to this were obtained in the case of lot 78G. Why this should be the case is not apparent.

Unfortunately it is not possible to tell from the available data on rats treated whether the increased number of distributions, and the change in the Permutit method, resulted in any increase in the potency of the extract preparations. Also, when tested, lots 678C and 678D were old. The poor response to extract 70B is not easy to explain although the rats used were of a small size.

In the following tables are indicated the solid contents of the extracts at various stages compared with figures which are the averages of five preparations taken from Swingle & Pfiffner (23).

Table V11.

Alcohol, Benzene and Acetone Stages.

Solid content of alcohol extracts in gm. per kgm.

Lot No.	60	62	64	66	67	68	70	Swingle & Pfiffner
1st alch.	28.4	25.8	29.6	30.4	28.0	30.1	34.2	
2nd alch.	8.47	8.96		15.5	14.1	14.2	13.1	
Benz. 1.	10.2	10.1	6.6					
2.	3.39	1.77	0.37					
3.	1.01	0.35	0.08					
4.	0.36	0.09	0.05					
Total	14.9	12.3	7.1	20.5	23.7	22.1	24.8	25.8
Acetone	2.83	2.69	2.38	3.88	3.88	4.42	8.00	6.3

Table V111.

70% Alcohol and Final Extract.

Lot Number	678B	678C	Swingle & Pfiffner
70% alcohol 1.	0.0972	0.1312	
2.	0.0783	0.0573	
3.	0.0594	0.0409	
4.	0.0444	0.0380	
5.	0.0394	0.0427	
Total	0.319	0.310	0.800
Final aqueous extract	0.054	0.054	0.072

The residue determinations represent in all cases the average of two duplicate determinations done by evaporating down an aliquot of the solution on the water bath and allowing to stand overnight in the dessicator before weighing. The Swingle & Pfiffner benzene solution contains a greater residue than does that from any of our own preparations, and from the tabulated values of the residues in each benzene extract in lots 60, 62, 64, it is seen that further extraction with benzene could not have increased the solid content to the quoted figure. Similarly the Swingle & Pfiffner acetone solution contains larger residue than any of our preparations with the exception of lot 70. In this case the phospholipin, due to a fault in the precipitation procedure, was not so completely removed as was generally the case. The residues in the combined 70% alcohol solutions are also less than that found by Swingle & Pfiffner as are also the final residues in the Seitz filtered aqueous solutions.

Why the residues in our extracts are consistently less throughout the process is difficult to say. Differences in the types of the animals from which the glands were taken in the two cases perhaps accounts for the discrepancies observed.

Following lot 71 no more extracts were prepared

according to the Swingle & Pfiffner method but several preparations were made following the directions of Kutz.

(2) Kutz method using acetone.

The following gives briefly the details of two preparations on the basis of 1 kgm. gland substance.

The gland substance of lot 72 was treated three times with acetone, first with 1.0 L. of 99% acetone for four hours, second with 1.0 L. of 96% for one hour, and then washed with 0.5 L. of 99% acetone.

By concentration under reduced pressure a total volume of 0.36 L. of aqueous solution was obtained. This aqueous concentrate was extracted with benzene in the usual manner. The benzene was then washed seven times with 26 cc. portions of 4% sodium bicarbonate after standing for 12 days in the refrigerator. After concentration to 32 cc. the benzene was washed four more times with 10 cc. of bicarbonate. The bicarbonate solutions were then washed with a portion of benzene which was afterwards combined with the main extract.

The benzene solution was subjected to distillation, water added and after removing the benzene completely an aqueous emulsion with a mass of emulsified fat was encountered. The emulsion was poured off and the fatty mass well laxivated with water two more times. To the watery emulsion were added 10 drops of glacial acetic

acid and allowed to stand in the refrigerator overnight. The following day it was found that the emulsion could not be broken by centrifuging but that after the portionwise addition of 0.5 cc. glacial acetic acid the fat could be filtered off using soft filter paper. By the careful addition of concentrated sodium hydroxide with agitation the solution was neutralized and the purplish-red extract Seitz filtered.

The preparation of lot 74 differed in the following ways from that of lot 72, for the initial extraction of the glands 2.0 L. of 98% acetone over a period of 8 hours were employed, the benzene solution was concentrated to approximately 120 cc. before washing 12 times with 37 cc. portions of sodium bicarbonate (4%), and once with 25 cc. of water, and no acetic acid was employed to precipitate the fats from the final aqueous solution. In this case it was found much easier to prepare the final aqueous solution of the extract on account of the much smaller fat content.

The activity of these two preparations was much greater than those prepared according to the Swingle & Pfiffner method as is shown by the rats in groups 24 and 25.

(3) Kendall method using acidified acetone.

One extract was prepared according to the directions of Kendall.

The gland substance was minced into 1.0 L. of 97% acetone to which was added 35 cc. of 5 N. sulphuric acid. This amount of acid served to bring the extract mixture to pH3. After a four day period of extraction the gland residue was separated and discarded. The acetone was removed from the dark brown extract under reduced pressure, the residual volume of the aqueous solution being 530 cc. To this solution was added hot saturated barium hydroxide to pH5.5 followed by 3.5 gm. lead acetate. The solution was then readjusted to pH5.5 by addition of more barium hydroxide.

The precipitate was separated by decanting and centrifuging and was washed with a small volume of water. The combined aqueous solutions were then concentrated under reduced pressure to a volume of 21 cc. to which was added 5.3 gm. lead acetate in hot saturated solution followed by 2.1 gm. solid sodium bicarbonate. The solution was then treated with 70 cc. of acetone containing 25% by volume of methyl alcohol.

After standing in the refrigerator overnight the solution was filtered and concentrated to a syrup. The syrup was extracted with 35 cc. acetone containing 25% absolute methyl alcohol, followed by 17 cc. of the same mixture used portionwise.

The acetone solutions were combined, filtered into

a mortar, and triturated with potassium carbonate and lead nitrate. On testing a portion of the supernant liquid with the uric acid reagent (Folin-Denis), it was found to contain a relatively large amount of adrenalin. Accordingly 5% of water was added to the mixture in the mortar and the whole retriturated. The supernant fluid then contained very little adrenalin.

The solid removed from the solution by filtration was washed on the filter paper with a portion of acetone. Into the filtrate hydrogen sulphide was passed in excess, the precipitated sulphides were centrifuged off, washed, and after standing in the cold overnight the solution was taken down to dryness. The residue was extracted with 17 cc. of the acetone-methyl alcohol mixture, the acetone was removed and the extract introduced into aqueous solution.

On testing on a group of rats (26) this extract was found to be quite inactive when given at the levels of 75 and 50 gm. of whole glands per day.

From the gland residue of lot 72 were prepared two extracts according to the method of Kendall. These also turned out to be quite inactive when tested on rats at a high dosage level. Accordingly experimentation along these lines was discontinued.

(4) Grollman & Firor method.

The Grollman & Firor method is very similar to the

Kutz method of preparing adrenal extracts. These authors, however, advocate the use of dissected cortical material which they collect in the abbatoir and immediately throw into acetone cooled with carbon dioxide snow. Later the glands are minced in the still frozen condition into more acetone.

We have not found it convenient to take such elaborate precautions and have prepared extracts from gland substance such as has been used in the work previously reported.

The preparation of lot 78 is described on a basis of 1 kgm. gland substance.

The gland substance which had been stored frozen for four months was minced into 3.0 L. of 99% cold acetone and allowed to stand in the refrigerator three hours. The mixture was then heated to 40°C. in a water bath and after standing two hours was replaced in the refrigerator overnight.

The extracting mixture was warmed to room temperature and the gland residue removed.

The acetone solution was then subjected to distillation under reduced pressure. Inadvertently the volume was reduced to 810 cc. (Grollman & Firor 1,300 cc.) so the volume was made up to 1,220 cc. by the addition of 410 cc. 15% acetone. The extract was then filtered

using soft filter paper.

The aqueous acetone extract was then washed with seven 160 cc. portions of benzene. During this extraction process there was a reduction in volume of the aqueous acetone solution of 240 cc. The seven combined benzene solutions were then washed twice with 57 cc. portions of saturated sodium bicarbonate. The bicarbonate removes from the benzene solution a dark green pigment and leaves the latter clear yellow in colour. The two bicarbonate solutions were afterwards washed back with small volumes of 15% acetone in benzene. Eight cc. N. hydrochloric acid were added to the benzene solution, the extract well shaken and then centrifuged to completely remove the acid.

The benzene solution was then concentrated to a smaller volume, 20 cc. 1% saline added, and the benzene completely removed. A fatty residue remaining in the flask after decanting the aqueous solution was reextracted with water. This aqueous solution was added to the saline and the extract after neutralization made up so that 1 cc. contained the equivalent of 23 gm. of whole gland substance.

On injecting 1 cc. of this extract into the peritoneal cavity of a rat an irritating effect which passed off after half an hour was observed.

Rats (group 29) treated with this extract reacted in a most remarkable manner when the equivalent of 11.5 or 23 gm. of whole gland substance was injected per day. None of the rats died until 3.5 or more days after cessation of treatment and three out of eight continued to live. Accordingly a portion of the extract was diluted with water and given at the equivalent of 7.8 and 3.9 gm. whole gland substance per day. The rats on the lower dosage level died within 8.5 days in spite of the treatment but the others lived until the therapy was discontinued.

This extract, although made from gland material stored for four months, is by far the most potent yet tested. The hormone, therefore, was either not extracted from the gland substance in significant amounts by the methods previously used or was lost from the extracts at one or more points.

The method of preparation differed from those previously employed in several respects.

(a) The extraction was made with a larger volume of acetone.

(b) The gland solvent mixture was heated.

(c) The active material instead of being extracted from an unfiltered aqueous solution with benzene was extracted from a filtered aqueous acetone solution with benzene.

(d) Seven extractions with benzene instead of four or five were made.

(e) Only two bicarbonate washes were used.

(f) The benzene solution was washed with hydrochloric acid (N.).

(g) The final extract was in 1% sodium chloride instead of water.

It appeared most probable that the success of the Grollman & Firor method was to be attributed to the large numbef of benzene extractions made from the dilute acetone solution. An experiment was therefore made to test this hypothesis.

From the gland substance of lot 79 was prepared an aqueous acetone concentrate (1,300 cc.) similar to that of lot 78. After filtering this aqueous acetone solution it was divided into three equal parts which were treated as follows; (Figures all refer to 1 kgm. gland substance).

(1) Treated as lot 78 (79G).

(2) Further concentrated (190 cc.) to remove all the acetone and extracted five times with 50% acetone in benzene (79SG).

(3) As (2) but extracted four times with benzene (79S).

To the aqueous solution from (3) was then added 120 cc. acetone and 70 cc. benzene. The benzene-acetone extract was removed and the aqueous acetone solution extracted twice with 88 cc. portions of 50% acetone in benzene and the same number of times with 88 cc. portions of 33% acetone in benzene (79SR).

Each of the four extracts was then washed with two portions of saturated sodium bicarbonate solution and one of N. hydrochloric acid, concentrated and introduced into 0.8% sodium chloride.

These extracts were all found to be active when tested on rats (groups 32-36), although one or more of the rats in each group behaved inconsistently. The extracts 79G and 79Gs appear to possess approximately the same order of activity but 79S is less active than either. The most notable finding, however, was that 79SR contained considerable activity. Since this preparation was made from an aqueous solution which has previously been extracted four times with benzene, doubt is thrown on the efficacy of benzene extraction to remove the hormone from aqueous solution.

The results of the previous experiment makes it appear that the hormone is more soluble in acetone-benzene mixtures than in benzene. It was thought, that increasing the concentration of acetone in the benzene from 50 to 65% this difference in solubility might be further increased. Also, it seemed possible that filtering the aqueous concentrate might have resulted in appreciable loss.

Two extracts 83A and 83B were prepared from equal portions of an unfiltered aqueous concentrate. In the one case (83A) the concentrate was extracted with 65% acetone in benzene, and in the other (83B) with benzene alone. As the aqueous solution was washed with 65% acetone in benzene it was found that instead of obtaining

one greenish brown benzene wash followed by benzene washes containing decreasing amounts of green pigment as in lots 78 and 79, no clear green washes were obtained. Instead the washes were all a dirty brownish yellow colour.

Both solutions following the benzene stage were treated as the previous lots to prepare aqueous extracts. However, the two preparations were found to be toxic when injected intraperitoneally into normal rats. Unfortunately, in attempting to get rid of the toxic material the major portion of the activity was either destroyed or lost. It was concluded from the difficulty experienced that it is advisable to filter the aqueous solutions and that 65% acetone-benzene is not a good agent for extracting the hormone from aqueous solution.

The extraction of the hormone from the aqueous solution might concievably be improved by the addition of a neutral salt. In order to test this possibility lot 86 was treated to obtain a filtered aqueous concentrate which was divided into three portions.

(1) Washed with 50% acetone in benzene (86A).

(2) Washed with 50% acetone in benzene after saturation with sodium sulphate (86B).

(3) To the aqueous solution was added 50% of acetone, saturated with sodium sulphate and sodium bicarbonate and washed with 50% acetone in benzene (86C).

of this solvent per kgm. gland substance. The alcohol, which was heated to 37°C. and allowed to cool during the extraction, was distilled to 200 cc. and washed five times with 66% acetone in benzene without filtering. The combined benzene washes were treated four times with small volumes of saturated sodium bicarbonate and with N. hydrochloric acid once. After concentrating to 7 cc. the benzene solution was poured into 150 cc. of cold acetone. The acetone solution was decanted, the precipitate washed, and the combined acetone solutions introduced into aqueous emulsion. By the addition of acetic acid the fat was precipitated from this emulsion and removed. The extract was then neutralized and tested on the rats in group 49.

The rats were fairly well maintained by this extract, yet it is not indicated that it is preferable to use alcohol as the initial extracting agent.

A preliminary experiment was also done to examine the effect of autolysing gland substance before extraction. The glands in lot 85 were minced into a small volume of water (360 cc.) and autolysed at 37°C. for 24 hours. Five litres of 99% acetone were then added and the gland substance extracted for a period of five hours. The acetone was then removed and the batch carried through as lot 84. The final aqueous solution had a slight

irritating effect on injection but this passed off in from 3 to 5 minutes.

Two rats in group 49 responded to this extract when the equivalent of 16 gm. gland substance was supplied per day but two others failed on 8 gm. Autolysis did not result in great destruction of the hormone.

The acidified aqueous solutions of lots 78 and 79 were found to give precipitates with phosphotungstic acid. Therefore, an experiment, using a portion of the final aqueous solution of lot 79 was performed to see if the hormone could be precipitated as a phosphotungstate and recovered without decomposition, or alternatively whether impurities could be removed in this manner. Very inconclusive results were obtained so the experiment was repeated using a portion of the benzene solution of 86A.

The benzene solution was concentrated and a small amount of phospholipin precipitated by pouring into cold acetone. From the acetone the extract was transferred into water and an equal volume of normal sulphuric acid added. The precipitated fat was removed and the acid solution allowed to stand overnight in the refrigerator.

To the cold extract was then added a small excess of 20% phosphotungstic acid. The precipitate after settling for two hours was then centrifuged off and dissolved in a small volume of 40% acetone to give a

pale brown solution. A small brown residue failed to dissolve. The phosphotungstates were then reprecipitated by pouring the acetone solution into 10 volumes of 0.5 N. sulphuric acid, allowed to settle, and redissolved in 40% acetone. Barium hydroxide was added dropwise to this solution to pH8, (excess $Ba(OH)_2$ present) and the barium phosphotungstate immediately filtered off. By the addition of sulphuric acid the extract was brought back to the acid side of neutrality and the barium in solution completely removed. After removing the acetone and making up to volume this extract fraction (86P) was Seitz filtered.

The centrifugate from the phosphotungstates was similarly treated cautiously with barium hyfroxide to remove the excess of acids, filtered, and brought back to approximately pH6.5 by the addition of sulphuric acid. This fraction (86PS) was then Seitz filtered after making up to volume.

These two extracts were tested on the rats in group 51 and both proved to be quite inactive when given at the dosage level of 35 gm. gland equivalent per day. Equal portions of the two extracts were mixed and given at the same dosage level but entirely negative results were again obtained. The treatment either destroyed the hormone or it was lost from the extract at some stage.

C. Extracts Provided for Clinical Use.

Many of the extracts tested on the experimental animals, and others specially prepared for the purpose have been used in the treatment of patients with Addison's disease.

The results obtained in one typical case, W. B., have been reported by Cantor & Scott (31,33).

This patient was treated with extracts made according to the Swingle & Pfiffner method using both acetone and alcohol as the initial extracting agent.

Lot 50 and 51 served to maintain W. B. when given at the rate of 210 gm. and 180 gm whole gland equivalent per day. When given to cats 28 and 30 at a dosage level from 100-500 gm. per day no response was evident. Similarly, at a later date, the patient was maintained over a period of two months on lot 69 using a dosage of 80 gm. per day. This lot was prepared using alcohol as the initial extracting agent and the Swingle & Pfiffner method except that the adrenalin was partly removed from the benzene aolution by washing with 4% sodium bicarbonate.

Although this extract was never tested on adrenalectomized cats it is improbable that this dosage would have maintained an animal. The hormone requirement of the patient was apparently relatively small.

The remissions induced by withholding extract

treatment showed clearly that the patient was actually suffering from a cortical hormone deficiency and that the extracts contained the necessary material.

A patient, M. H., under Dr. Weinlos (supervision of Dr. Scott) with typical symptoms of Addison's disease was treated with lot 678 over a period of three weeks. During the latter part of this period the patient received 80 gm. gland equivalent per day and on discharging was subjectively much improved.

More recently a patient, McB., under Dr. Scott, has been treated with extracts prepared according to the Grollman & Firor method. In order to maintain this patient it has been necessary to administer the equivalent of 100 gm. gland substance per day and during a period of relapse a much larger dosage was necessary, 250 gm. being supplied per day. Since extracts made according to the Grollman & Firor method are very active when tested on rats it appears that the hormone requirements of this patient is very much greater than that of W. B.

Summary.

1. Potent extracts, as tested on cats, can be prepared by the Swingle & Pfiffner method.
2. Adrenalectomized cats required of our preparations at least two to four times the dosage used by these authors.
3. As tested on adrenalectomized rats more potent extracts can be prepared following the relatively simple procedure of either Kutz or Grollman & Firor.
4. Experiments done, although inconclusive, indicate that the hormone is more readily extracted from a crude aqueous concentrate by 50% acetone in benzene than by benzene.
5. Some patients with Addison's disease respond to extract treatment.

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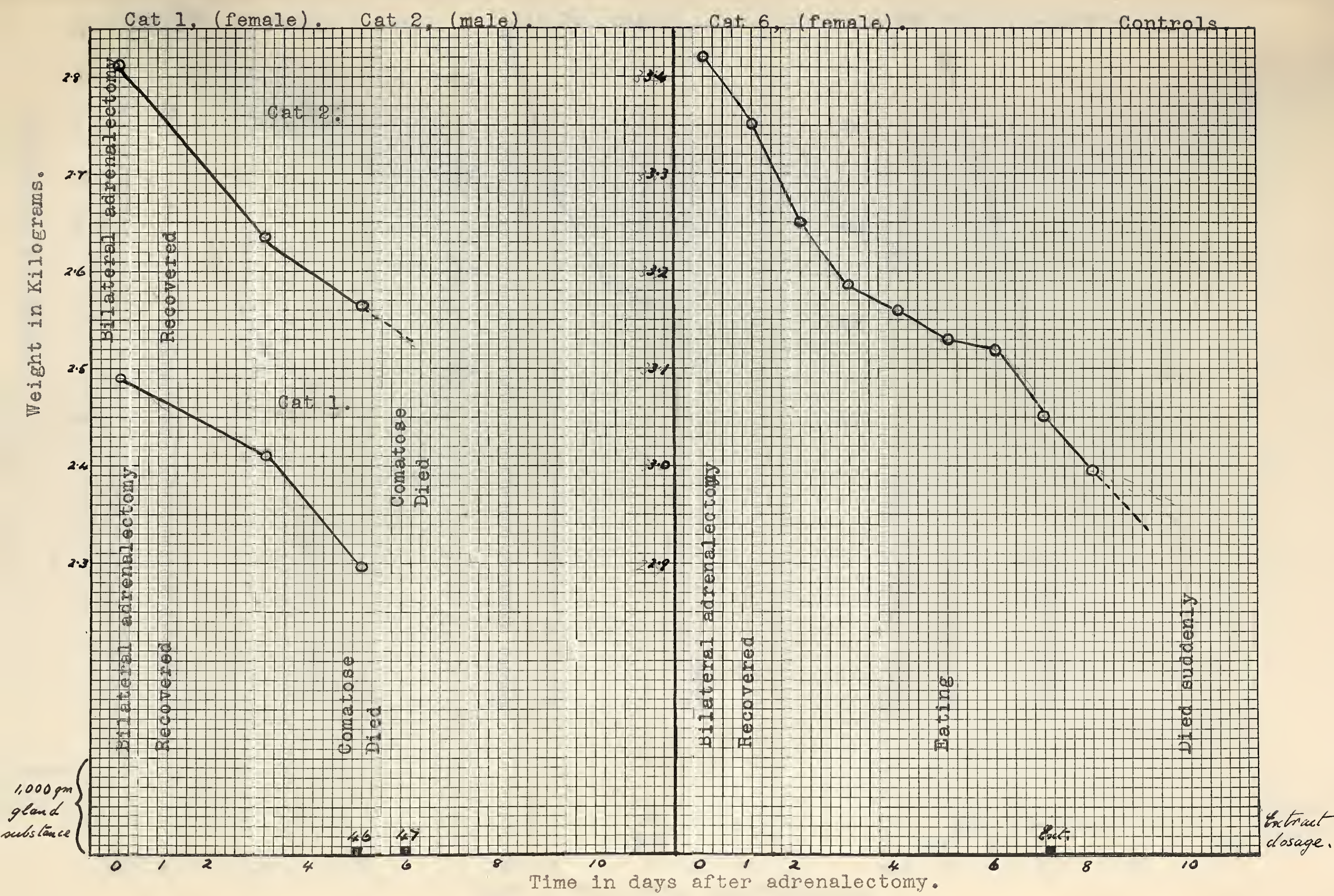
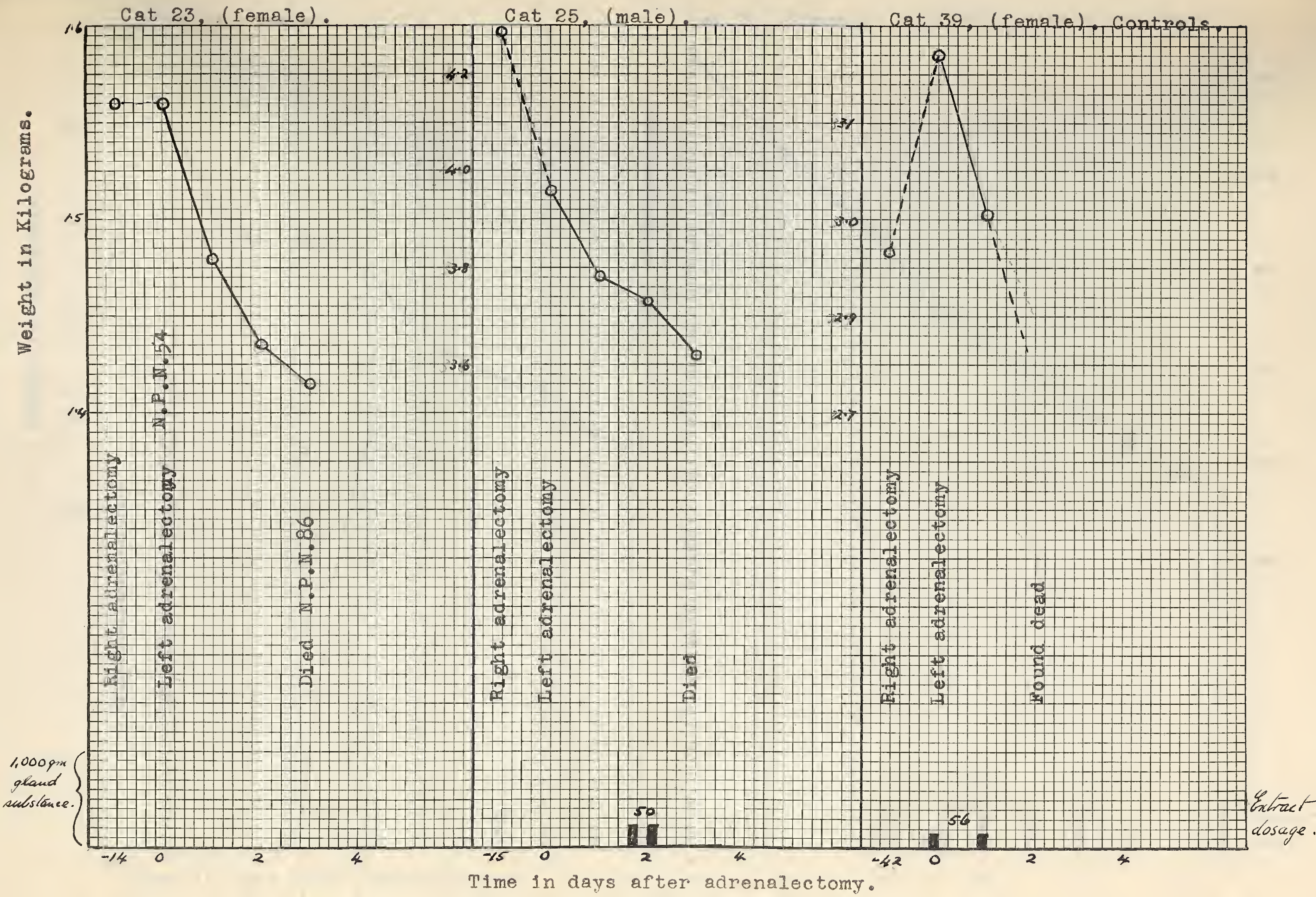
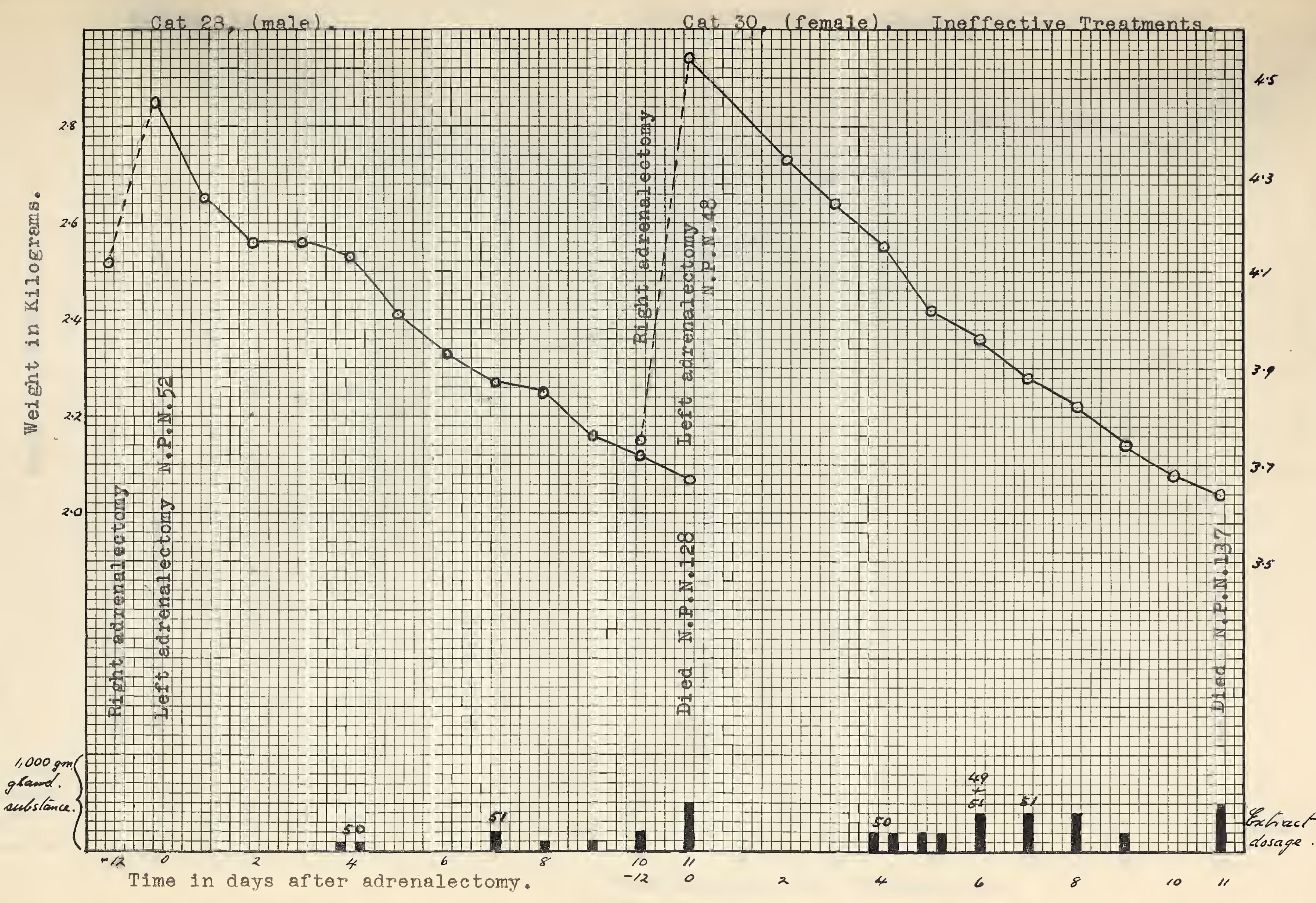
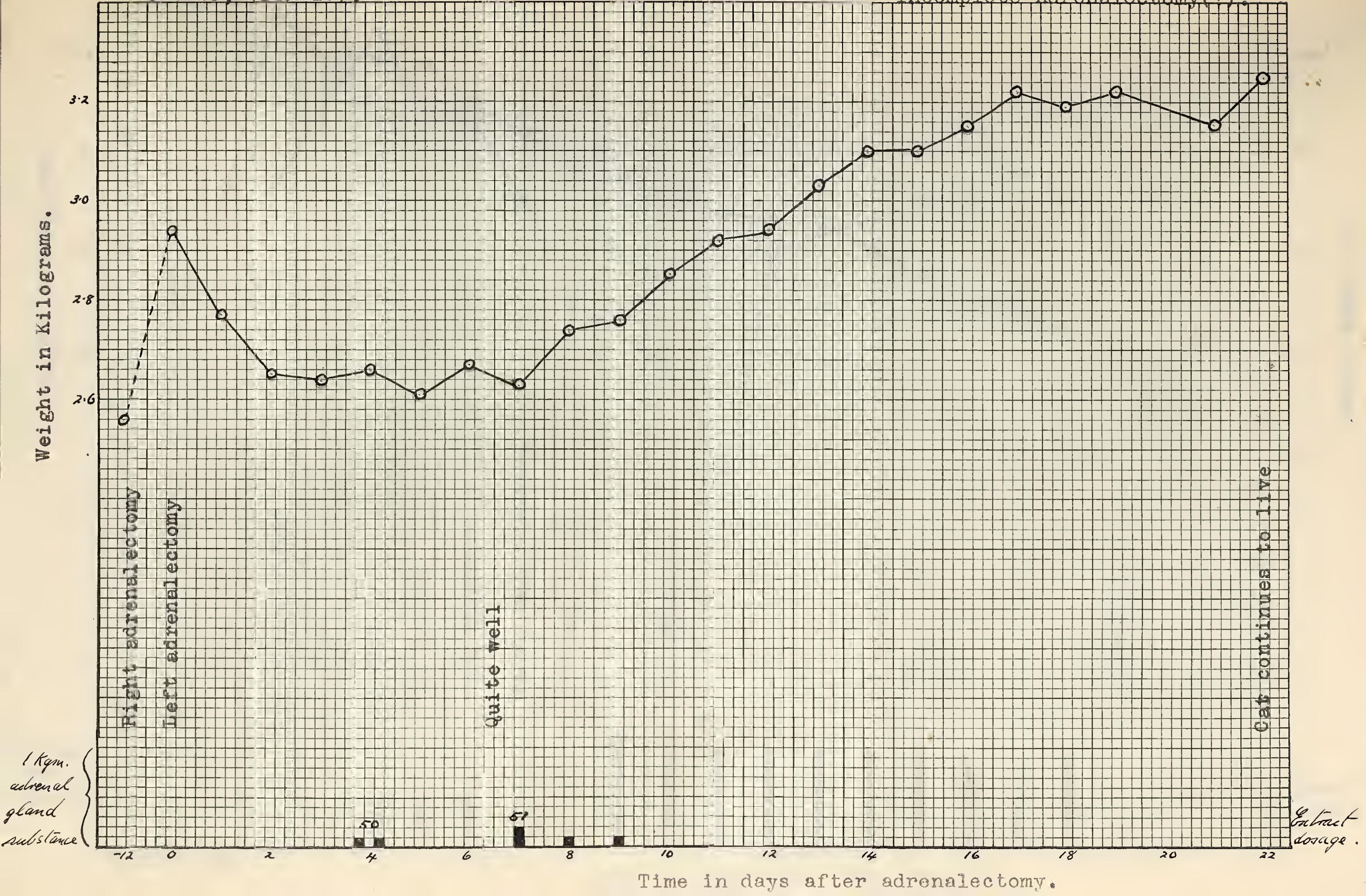
Protocols.


Cat 1, (female).
Cat 2, (male).
Cat 6, (female).
Controls.
Cat 2.
Cat 1.
Weight in Kilograms.
2·8
2·7
2·6
2·5
2·4
2·3
3·4
3·3
3·2
3·1
3·0
2·9
Bilateral adrenalectomy
Recovered
Comatose
Died
Eating
Died suddenly
1,000 gm gland substance
46
47
Ext.
Extract dosage.
0
1
2
4
6
8
10
Time in days after adrenalectomy.

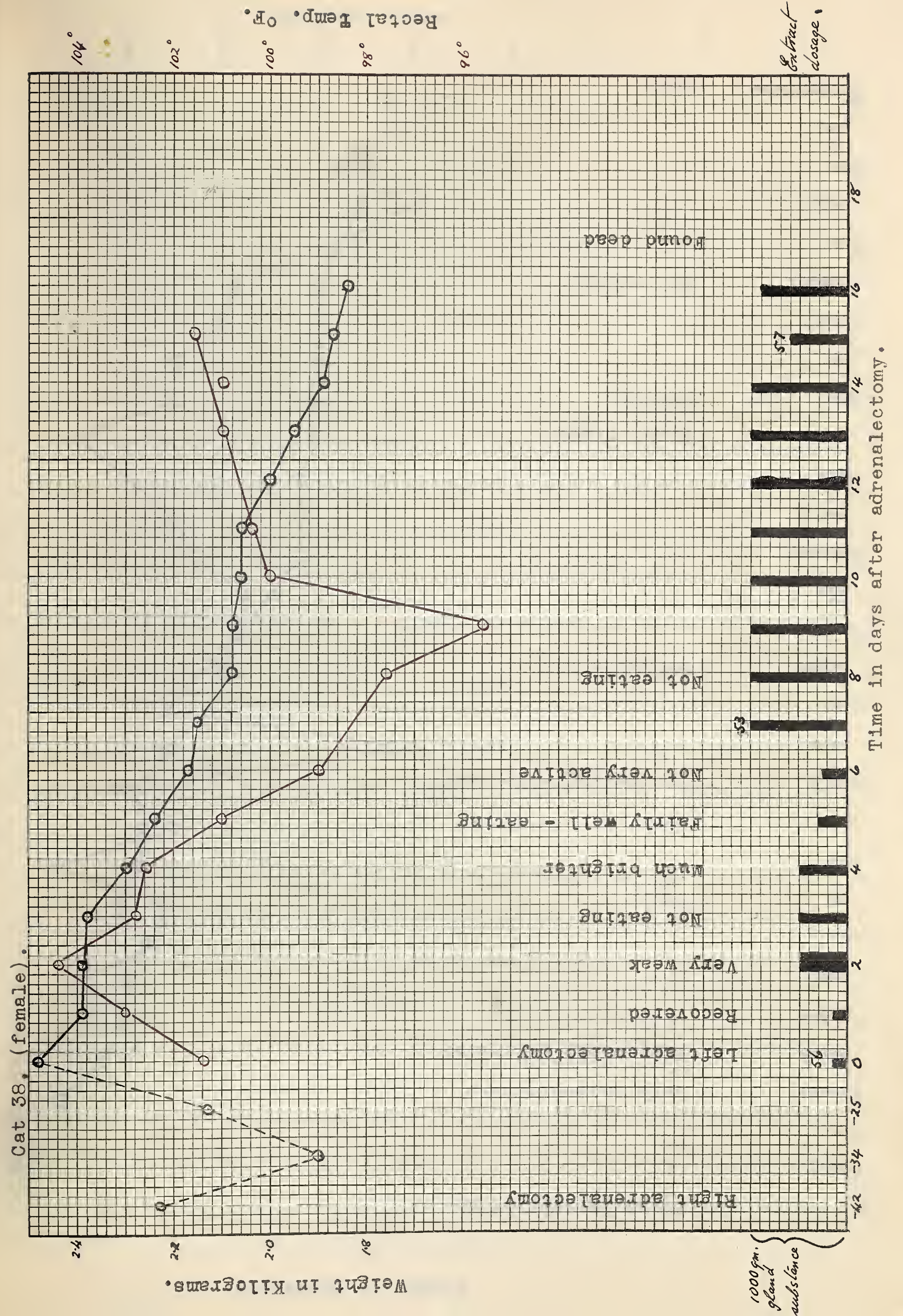

Cat 23, (female).
Cat 25, (male).
Cat 39, (female). Controls.
Weight in Kilograms.
1.6
1.5
1.4
4.2
4.0
3.8
3.6
3.1
3.0
2.9
2.7
Right adrenalectomy
Left adrenalectomy
N.P.N.54
Died N.P.N.86
Right adrenalectomy
Left adrenalectomy
Died
Right adrenalectomy
Left adrenalectomy
Found dead
1,000 gm gland substance.
50
56
Extract dosage.
-14
0
2
4
-15
0
2
4
-42
0
2
4
Time in days after adrenalectomy.


Cat 28, (male).
Cat 30, (female). Ineffective Treatments.
Weight in Kilograms.
2·8
2·6
2·4
2·2
2·0
4·5
4·3
4·1
3·9
3·7
3·5
Right adrenalectomy
Left adrenalectomy N.P.N.52
Right adrenalectomy
Left adrenalectomy
N.P.N.48
Died N.P.N.128
Died N.P.N.137
1,000 gm. gland. substance.
Extract dosage.
50
51
50
49 + 51
51
-12
0
2
4
6
8
10
11
-12
0
2
4
6
8
10
11
Time in days after adrenalectomy.

Cat 29, (female). Incomplete Adrenalectomy(?).



Cat 38, (female).



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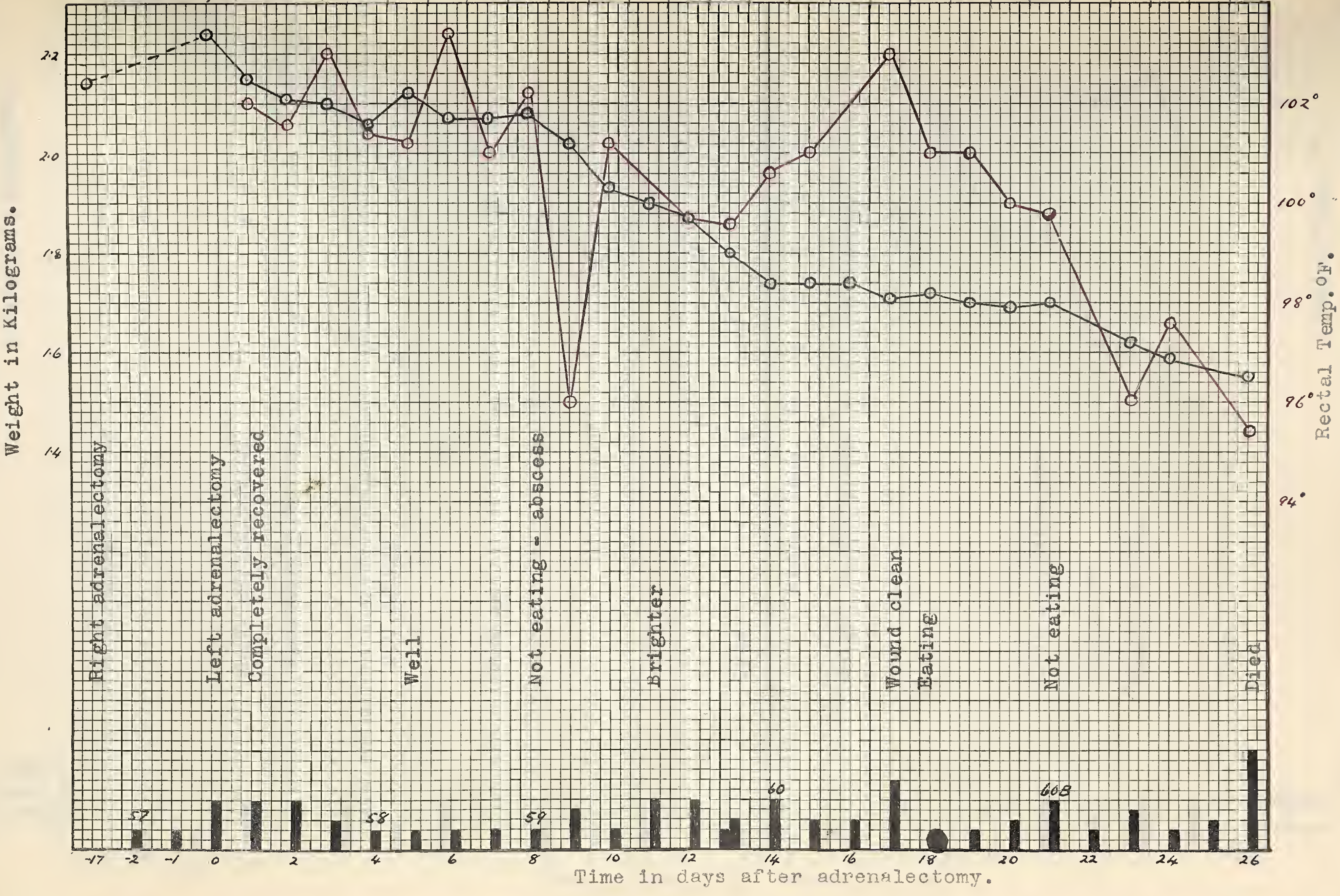
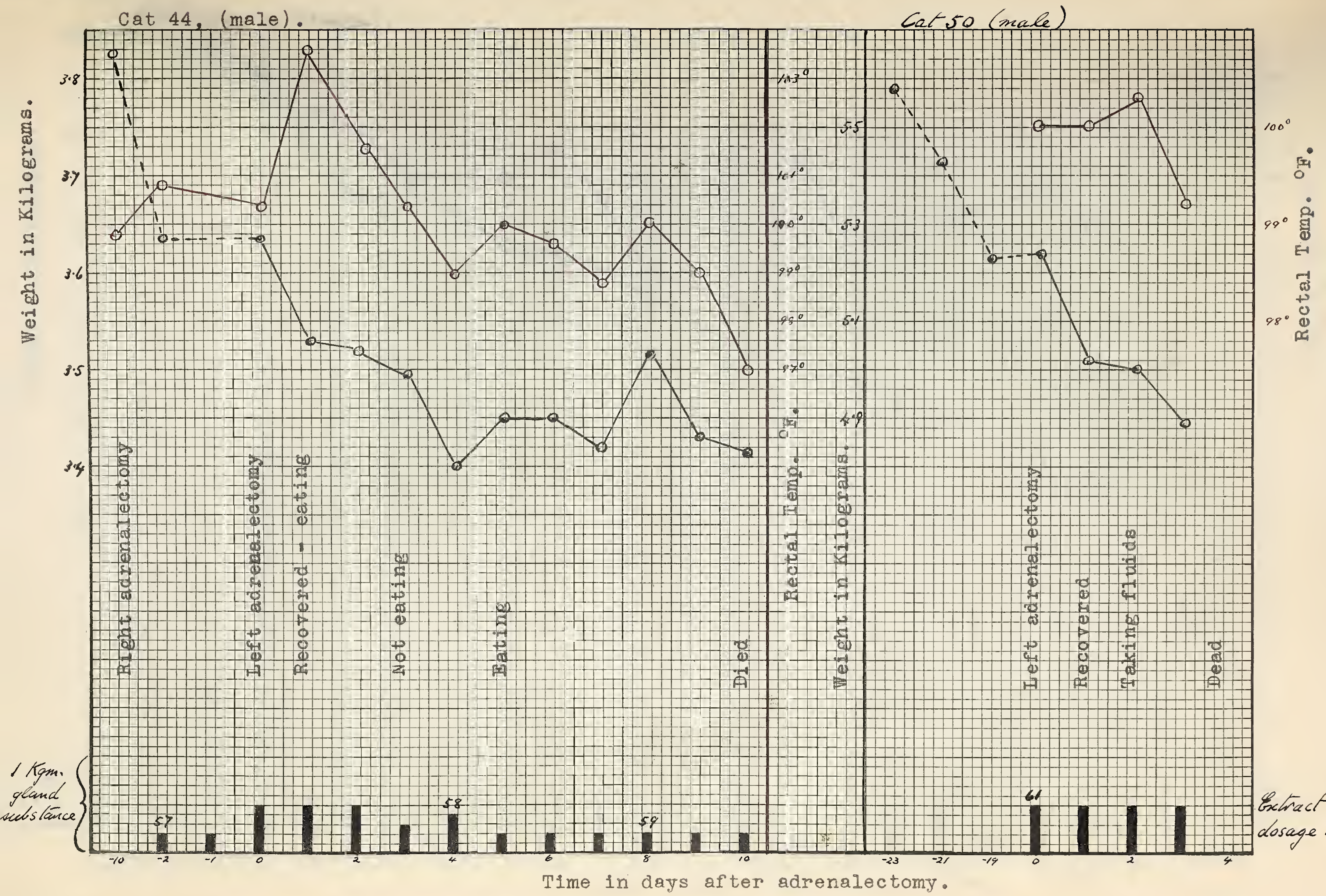
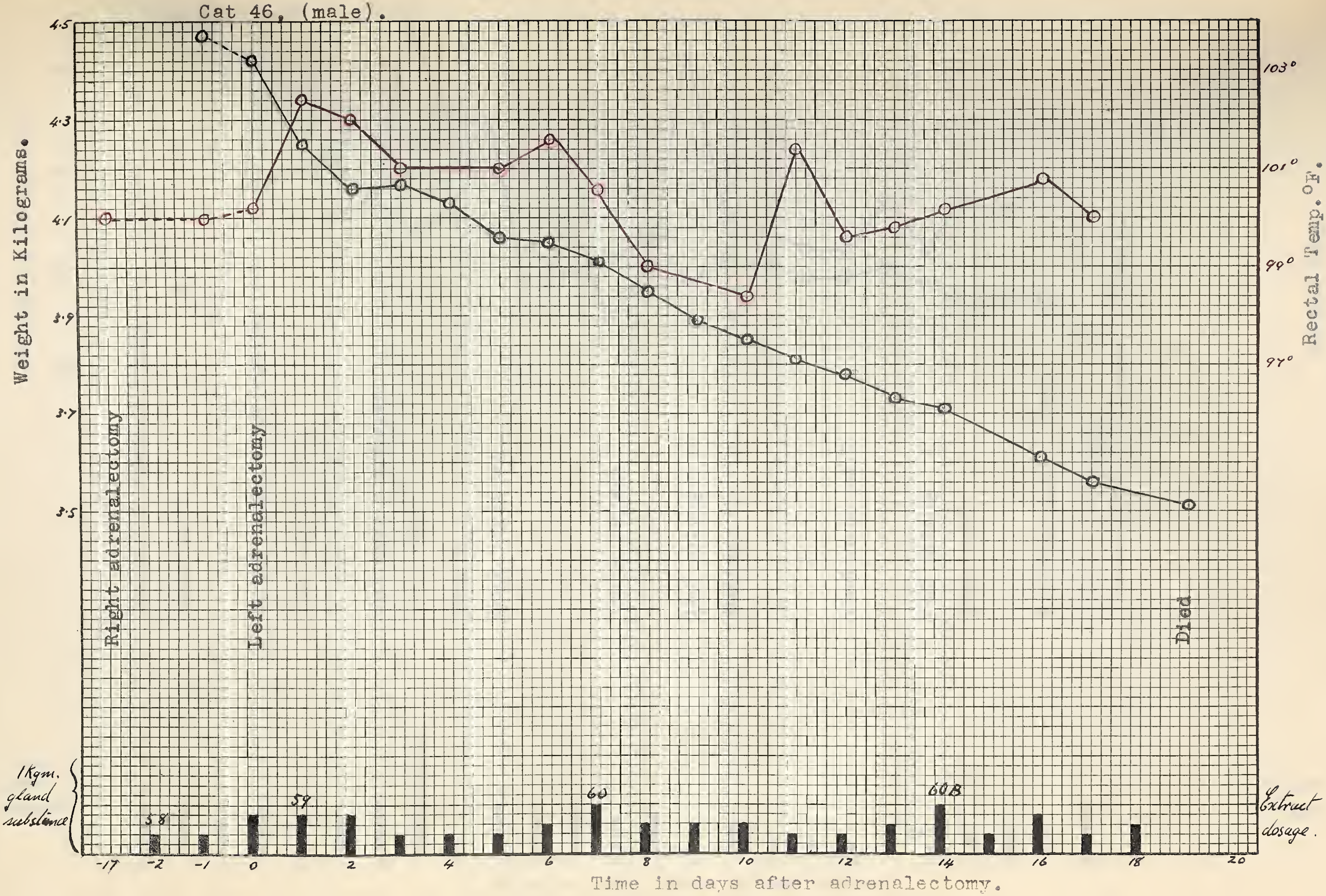
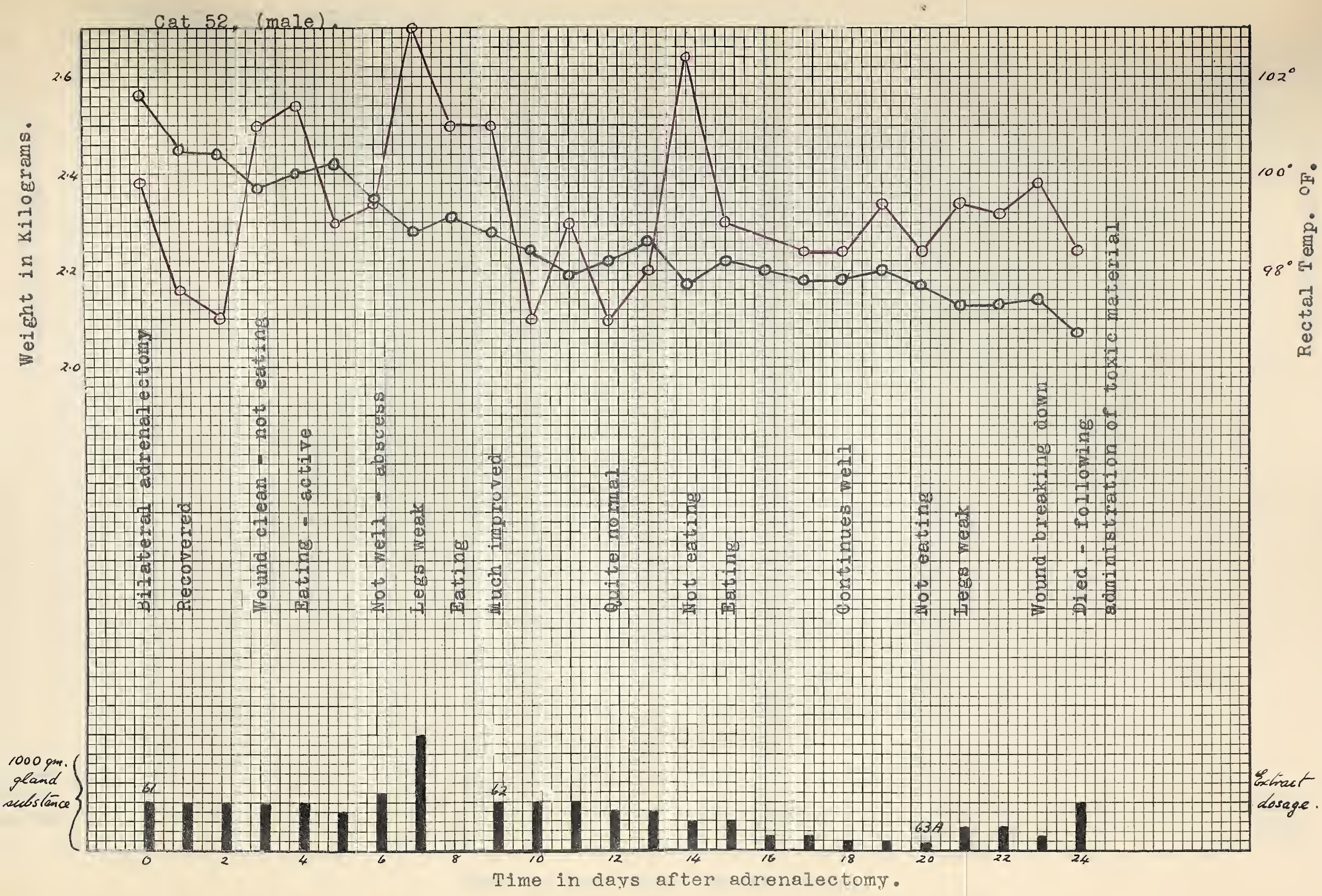


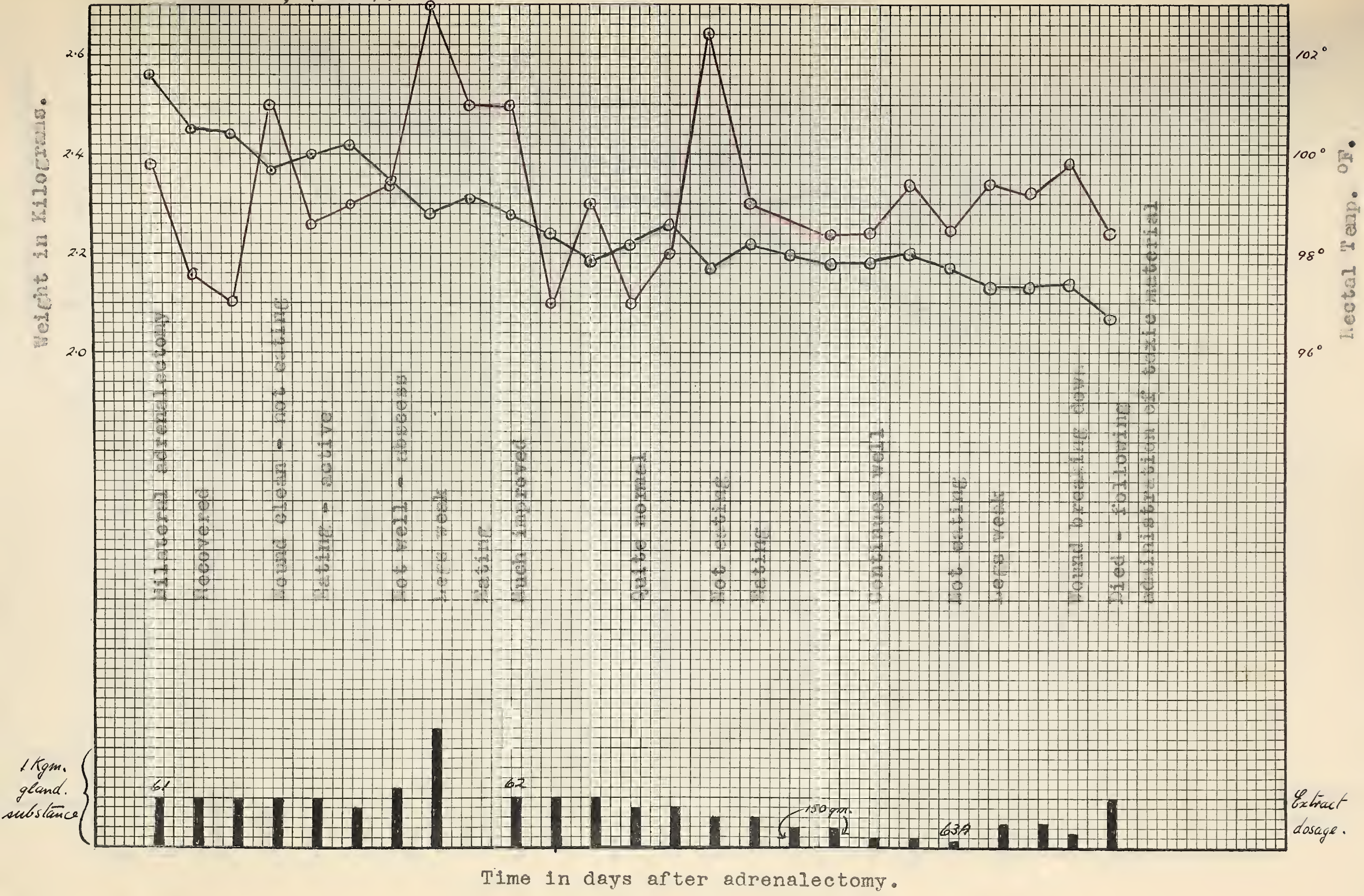

Cat 44, (male).
Cat 50 (male)
Weight in Kilograms.
3·8
3·7
3·6
3·5
3·4
Rectal Temp. °F.
103°
101°
100°
99°
98°
97°
Weight in Kilograms.
5·5
5·3
5·1
4·9
Rectal Temp. °F.
100°
99°
98°
Right adrenalectomy
Left adrenalectomy
Recovered - eating
Not eating
Eating
Died
Left adrenalectomy
Recovered
Taking fluids
Dead
1 Kgm. gland substance
Extract dosage
57
58
59
61
-10
-2
-1
0
2
4
6
8
10
-23
-21
-19
0
2
4
Time in days after adrenalectomy.

Cat 46, (male).

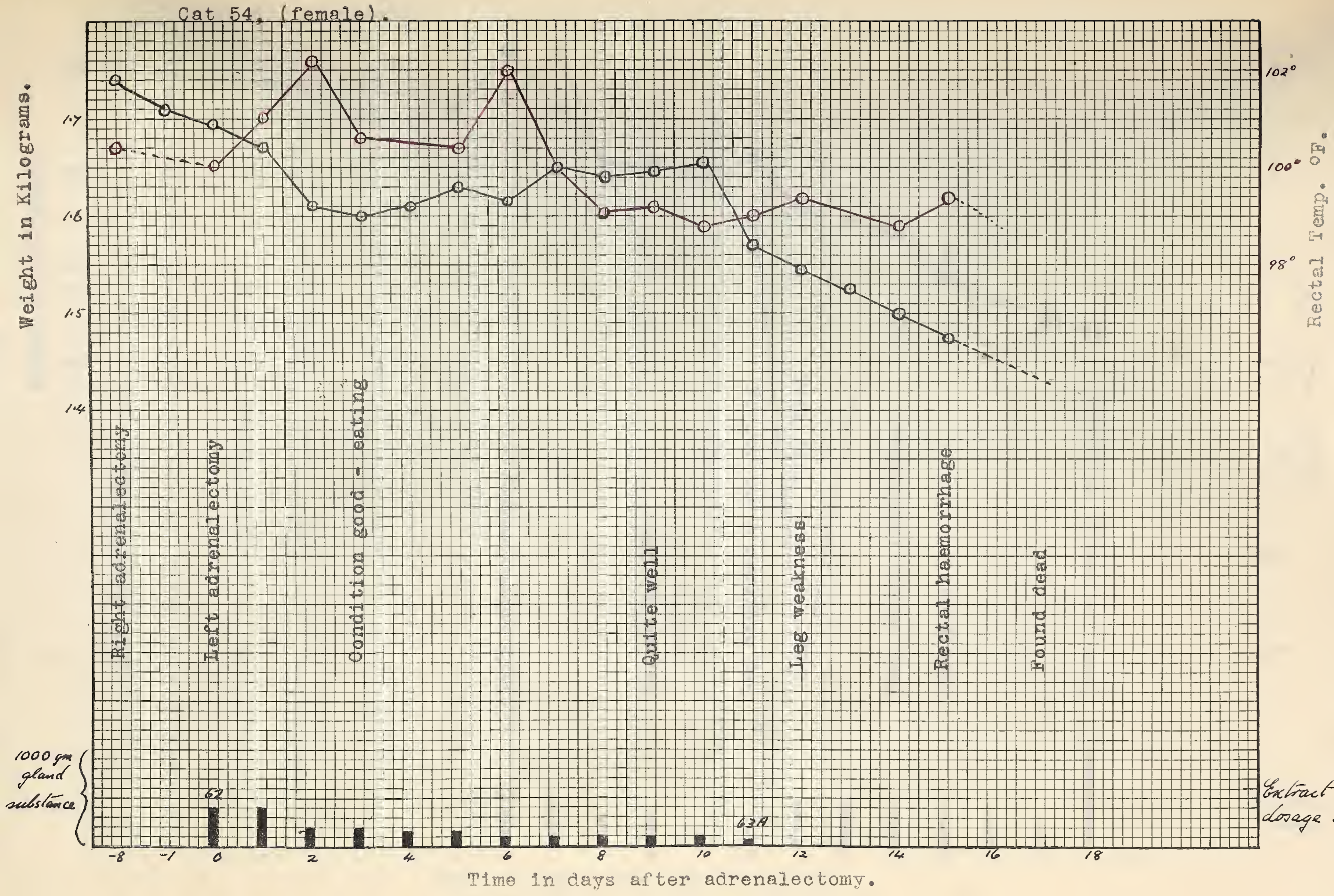

Weight in Kilograms.
4.5
4.3
4.1
3.9
3.7
3.5
Rectal Temp. °F.
103°
101°
99°
97°
Right adrenalectomy
Left adrenalectomy
Died
1 Kgm. gland substance
Extract dosage.
58
59
60
60B
-17
-2
-1
0
2
4
6
8
10
12
14
16
18
20
Time in days after adrenalectomy.

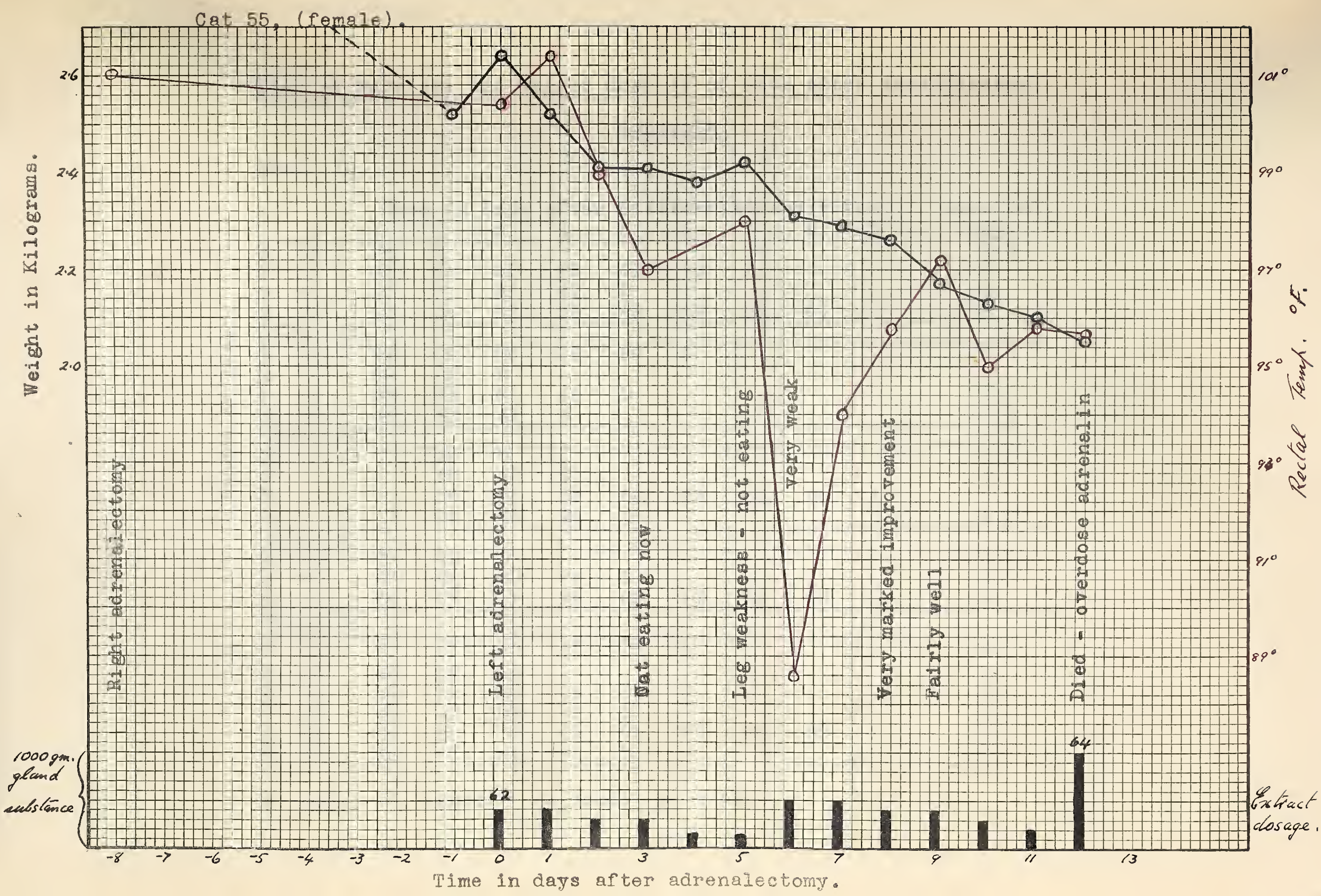

Cat 52, (male).
Weight in Kilograms.
2.6
2.4
2.2
2.0
Rectal Temp. oF.
102°
100°
98°
Bilateral adrenalectomy
Recovered
Wound clean - not eating
Eating - active
Not well - abscess
Legs weak
Eating
Much improved
Quite normal
Not eating
Eating
Continues well
Not eating
Legs weak
Wound breaking down
Died - following administration of toxic material
61
62
63A
1000 gm. gland substance
Extract dosage.
0
2
4
6
8
10
12
14
16
18
20
22
24
Time in days after adrenalectomy.

Cat 52, (male).


Weight in Kilograms.
2.6
2.4
2.2
2.0
Rectal Temp. °F.
102°
100°
98°
96°
Bilateral adrenalectomy
Recovered
Wound clean - not eating
Eating - active
Not well - abscess
Lets weak
Eating
Much improved
Quite normal
Not eating
Eating
Continues well
Not eating
Lets weak
Wound breaking down
Died - following
administration of toxic material
1 Kgm. gland. substance
61
62
150 gm.
63A
Extract dosage.
Time in days after adrenalectomy.

Cat 54. (female).




Cat 55, (female).

Time of Survival and Weight Changes of Rats after Adrenalectomy.

Controls.

Group 19.

Weight in grams.

	Time in Days after Bilateraladrenalectomy.										Survival.
	0	1	2	3	4	5	6	7	8	9	Days
M	53	54	D								1.5
F	65	64	64	62	62	61	60	D			6
F	68	67	67	63	62	D					4
M	95	95	94	92	92	88	88	84	81	D	8
Group 20.											
M	92	91	89	89	89	89	D				5.5
M	70	69	65	D							3
F	65	65	63	61	59	D					4.5
F	46	45	43	41	D						3.5
Group 24.											
F			40	D							2.5
M			48	42	D						3.5
M			58	53	59	60	61	65	66	A	A
F			46	42	42	D					4.5
Group 27.											
M	91	97	93	D							2.5
M	87	87	84	80	76	D					4.5
M	70	76	77	77	78	82	85	89	89	A	A
Group 40.											
F	58	57	57	55	55	54	D				5.5
M	59	58	58	54	D						3
F	63	60	60	56	55	53	D				5.5
F	58	56	56	52	50	D					4.5
M	82	80	81	76	75	78	79	79	80	D	8
M	67	66	67	61	60	D					4

M, male; F, female; D, dead; A, alive.

Controls.

Group 36.   Low Salt Diet.

Weight in grams.

	Time in Days after Bilateral Adrenalectomy. 0	1	2	3	4	5	6	7	8	9	Survival Days.
M	80	78	78	77	72	D					4.5
F	91	86	87	85	80	77	73	73	D		7
F	75	76	80	84	86	90	97	99	95	105	A

Group 38.   Low Salt Diet.

	0	1	2	3	4	5	6	7	8	9	Survival Days.
F	80	79	79	74	68	D					4.5
M	71	71	73	71	61	61	D				5
M	53	51	D								1.5
M	65	61	63	60	55	53	D				5

Rats Treated With Extracts 54, 55, 56.

Weight in grams.

Group 6. Extact 54.

Time in Days after Bilateral Adrenalectomy.

	0	1	2	3	4	5	6	7	8	9	10	11	12	13	Survival Days.
F	85		73	70	66	63	59	D							6.5
F	96		87	83	D										3.5
M	100		89	86	D										3
M	110		98	94	D										3.5
Dose				135	90	90									
Group 9. Extract 55.															
F.	112	114	113	112	107	108	105	105	100	100	101	104	102	98D	14.5
F	110	107	106	106	99	98	91	88	83	79	D				9
Dose					20	20	40	40	40	40	40	40	40		
Group 11. Extract 55 & 56.															
F	117	113	109	102	D										3.5
F	107	103	99	97	94	93	95	92	94	90	87	83	D		11.5
M	161	152	147	140	138	135	131	129	D						7.5
M	132	133	126	125	120	118	116	116	115	120	124	130	132	A	A
Dose(55)		80	40	80	80	80	80								
(56)								47	47	47	47	47			

Dose in gm. whole gland equivalent.

Rats Treated With Extracts 60Bb, 62, 63B, 64.

Weight in grams.

Group 15. Extract 60Bb.

	Time in Days after Bilateral Adrenalectomy.														Survival
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	Days.
F	110	104	101	99	98	96	95	D							6
M	115	111	105	102	101	99	97	93	93	D					8.5
M	150	140	138	136	132	131	127	D							6.5
M	122	117	118	118	118	118	116	118	D						7
Dose			40	40	40	40	80	160	160						
Group 15. Extract 63B.															
F	107	103	100	100	99	98	98	93	D						7.5
M	118	115	111	110	109	108	105	99	96	D					9.5
F	155	149	146	145	146	146	154	154	157	161	A				A
M	135	130	126	121	116	115	115	110	104	D					8.5
Dose			25	25	25	25	50	50	100	100					
Group 16. Extract 63B.															
F	108	104		99	99	99	97	96	91	90	89		D		11
F	92	93		93	95	95	96	102	106	108	113	117	119	A	A
Dose		50	50	50	50	75	100	75	100						
M	133	123		114	115	112	110	107	104	102	D				9.5
Dose		75	75	75	125	125	150	150							
Group 16. Extract 64.															
M	144	140		129	132	126	131	D							6.5
M	147	138		132	134	125	134	137	134	126	123	126		D	13
Dose		75	75	75	75	150	100	100	150	150					
F	107	103		95	95	89	D								5
F	99	96		85	84	78	D								5
Dose		50	50	50	50	100									
Group 14. Extract 62.															
F	95	90	88	84	81	77	D								5.5
M	102	98	96	93	89	84	80	D							6.5
Dose				100	100	100	100								

Dose in gm. whole gland equivalent.

Rats Treated With Extracts 678 and 70B.

Group 19. Extract 678C. Dose 75 and 38 gm.

Weight in grams.

	Time in Days after Bilateral Adrenalectomy.														Survival
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	Days.
M	87	89	92	97	103	105	104	109	109	114	115	116	119	119	A
M	68	67	68	71	73	73	71	68	D						7.5
F	84	82	85	84	88	88	88	88	88	90	91	93	97	98	A
F	78	78	79	80	80	75	76	74	75	77	81	83	90	90	A
M	84	81	86	86	87	85	87	87	84	80	81	83	82	82D	13.5
M	70	68	68	65	65	60	58	D							6
F	90	86	88	85	82	77	78	73	70	D					8
F	84	83	82	77	75	73	D								5.5
Group 22. Extract 678D. Dose 50 and 25 gm.															
M		50	50	50	51	51	48	D							6
M		45	43	43	46	45	45	D							6
M		49	46	47	47	46	D								5.5
F		47	45	45	45	45	D								5.5
Group 21. Extract 70B. Dose 50 and 25 gm.															
M		40	37	37	38	40	40	D							6
M		44	43	43	43	44	D								5.5
F		48	47	48	48	47	48	D							6
F		45	44	46	46	46	46	D							6
		48	45	46	46	D									4.5
		42	41	42	42	42	D								5.5

Horizontal lines divide rats into two dose groups - upper section on higher dose.
Dose in gm. whole gland equivalent per day.
Between vertical lines rats received extract.

Rats Treated with Extracts 72, 74 and 73.

Weight in grams.

Group 24. Extract 72. Dose 56 gm.

Time in Days after Bilateral Adrenalectomy.

	0	1	2	3	4	5	6	7	8	9	10	11	12	13	Survival Days.
F			48	45	50	50	51	53	55	55	54	53	D		11
M			49	46	50	50	53	57	60	59	60	64	66	67	A
F			56	51	55	54	54	58	59	58	55	D			10.5
M			55	50	54	55	56	59	61	61	62	D			10.5
Group 25. Extract 74. Dose 25 and 12.5 gm.															
M	66	66	63	64	62	60	D								5.5
F	63	64	63	62	62	61	63	64	66	70	70	A			A
M	75	75	74	74	75	70	70	70	66	D					8.5
F	666	67	67	65	67	65	66	65	63	D					8.5
F	59	59	57	55	55	D									4
M	70	68	65	63	63	D									4.5
M	70	70	69	67	65	D									5
M	72	72	70	68	68	68	67	66	65	63	63	-	-	-D	13.5
Group 26. Extract 73. Dose 75 and 50 gm.															
M	53	53	53	D											2.5
M	55	55	54	51	D										3.5
F	35	35	34	D											2.5
F	43	46	46	46	D										3.5
F	45	46	45	44	D										3.5

Horizontal lines divide rats into two dose groups - upper section on higher dose.
Dose in gm. whole gland equivalent per day.
Between vertical lines rats received extract.

Rats Treated With Extract 78.

Group 29. Dose 23 and 11.5 gm.

Weight in grams.

	Time in Days after Bilateral Adrenalectomy.														Survival
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	Days.
F	70	69	67	69	72	72	74	73	76	79	81	81	79	80D	21
M	62	60	60	62	64	63	70	71	72	74	78	81	84	87	A
F	61	61	61	62	64	64	65	64	64	65	67	67	65	64D	13.5
M	74	75	75	76	75	75	75	77	76	75	78	80	79	78D	18
F	63	59	58	59	58	61	62	62	63	65	67	70	69	68D	19.5
M	66	67	66	71	73	75	79	83	83	88	94	95	99	99	A
F	50	51	50	50	53	56	56	56	57	59	60	63	63	65D	13.5
M	59	57	57	58	61	63	67	68	67	70	73	76	77	81	A
Group 30. Dose 7.8 and 3.9 gm.															
M	97	96	95	92	91	90	93	92	91	94	90	90	90	91D	14.5
F	73	74	71	72	73	75	78	82	83	90	89	86	89	92D	25.5
M	55	54	53	52	52	54	55	54	53	D					8.5
F	61	60	58	54	52	53	51	50	50	D					8.5

Rats Treated With Extracts 79.

Group 32. Extract 79G. Dose 15 and 7.5 gm.

Weight in grams.

	Time in Days after Bilateral Adrenalectomy.														Survival
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	Days.
F	75	72	68	65	65	68	67	67	68	66	68	68	70	69D	15
F	89	87	87	84	85	89	93	95	97	96	103	104	109	115	A
F	54	55	54	49	49	51	50	50	50	48	48	D			10
M	83	85	84	81	80	84	87	84	84	82	86	88	91	94D	17
Group 33. Extract 79GS. Dose 14 and 7 gm.															
M	75	74	74	72	69	68	D								5.5
F	79	77	77	74	77	83	80	83	84	80	84	82	90	90D	20
F	71	69	68	67	67	71	70	70	69	68	71	72	72	74D	18
M	52	52	53	50	52	53	52	54	55	52	56	53	57	54D	14
GROUP 35. Extract 79S. Dose 15 and 7.5 gm.															
M	54	55	54	D											2
F	81	80	81	79	74	72	68	67	68	69	73	73	77	79	A
M	73	72	72	68	63	63	61	60	58	57	D				9.5
F	74	72	71	73	73	71	71	72	74	76	79	80	82	85D	23
Group 34. Extract 79SR. Dose 43 and 21.5 gm.															
M	84	83	85	83	82	85	81	87	88	92	94	95	97	97D	16
F	69	68	68	69	70	71	67	72	68	76	77	78	76	78D	17
F	76	73	74	74	74	73	75	77	79	81	83	79	80	80D	17

Rats Treated With Extracts 84, 85, and 86.

Group 49. Extract 84. Dose 16 and 8 gm.

	Time in Days after Bilateral Adrenalectomy.														Survival
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	Days.
M	118	122	122	117	118	119	118	117	115	111	107	D			10.5
F	74	75	72	69	65	67	66	64	63	D					8.5
F	86	87	90	87	85	91	91	90	90	88	88	89	89	89D	13
M	98	101	101	100	98	104	100	100	100	97	94	94	D		11
Group 49. Extract 85. Dose 16 and 8 gm.															
F	100	98	96	90	86	88	89	87	86	84	81	81	D		11.5
F	87	89	87	86	82	85	85	85	84	85	85	87	88	87D	13
M	79	79	77	74	70	D									5
F	64	64	64	60	57	D									4.5
Group 51. Extract 86PS. Dose 35 and 17.5 gm.															
M		64	64	62	D										3
F		58	58	56	53	D									4.5
F		80	80	80	77	D									5
M		57	46	45	D										3
M		53	53	D											2.5
M		44	D												2
Group 51. Extract 86P. Dose 35 and 17.5 gm.															
M		81	81	80	78	76	72	D							6
M		64	63	62	59	58	D								5
M		77	76	76	73	D									4
F		69	68	67	64	D									4.5
F		72	71	70	67	65	64	D							6
Group 52. Extract 86P 86PS. Dose 35 gm.															
M		78	79	79	79	75	76	73	D						7
F		69	68	68	66	62	61	D							6
M		73	73	73	71	70	68	D							6

Rats Treated With Extracts 86.

Group 50. Extract 86A. Dose 15 and 7.5 gm.

Times in Days after Bilateral Adrenalectomy.

Weight in grams.

	0	1	2	3	4	5	6	7	8	9	10	11	12	13	Survival Days.
M	78		75	75	77	74	D								5.5
M	80	80	80	81	81	82	80	82	83	83	85	87	86	85D	13.5
M	95	96	95	98	99	100	99	100	104	107	106	105	98	D	12
M			69	69	69	68	66	D							6
F	79	75	75	76	77	78	75	77	75	72	72	73	70	D	12
F	71	67	69	66	68	68	68	68	72	71	73	77	74	74D	21
Group 50. Extract 86B. Dose 15 and 7.5 gm.															
F	63	63	63	62	63	D									4.5
M	81	82	83	84	86	89	90	91	95	95	98	103	101	100D	14.5
F	68	67	67	67	68	69	69	69		D					8
F	49	47	48	47	47	47	D								5.5
M	100		99	101	104	106	109	109	114	114	115	121	119	122	A
M		76	75	74	72	72	69	70	D						7.5
Group 51. Extract 86C. Dose 14, 7 and 3.5.															
F		60	59	56	54	D									4
M		51	53	51	D										3
M		56	56	54	D										3
M		62	61	61	D										3
F		53	52	51	48	D									4
M		74	73	72	70	D									4
F		65	65	63	60	D									4.5
M		63	63	62	59	D									4
F		81	81	83	80	78	77	D							6.5

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